ARTERIAL STIFFNESS AND HAEMORRHAGE IN THE VULNERABLE CAROTID PLAQUE

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Abstract

Background

Magnetic resonance imaging of carotid plaque haemorrhage (MRIPH) has recently been shown to be more superior than the degree of stenosis in predicting ischaemic stroke. Recently, mechanical factors such as arterial stiffness have also been suggested to be associated with stroke. Pulse wave velocity (PWV) is a non-invasive imaging technique to assess arterial stiffness and studies have shown that aortic PWV is an independent predictor of cardiovascular and cerebrovascular morbidity as well as mortality. The aims of this thesis were to test the feasibility of assessing carotid PWV using magnetic resonance imaging (MRI), to examine the association between carotid PWV and the degree of stenosis as well as the association between the status of MRIPH and carotid PWV.

Methods

29 patients (55 carotid arteries) with at least 50% unilateral carotid artery stenosis and 8 healthy volunteers (16 carotid arteries) were included in the study. PWV was derived from cine phase contrast using MRI at 3 Tesla. Carotid PWV was compared between the two groups, the relationship between carotid PWV and the degree of carotid stenosis as well as the presence of MRIPH were examined. All multiple linear and logistic regression analyses were adjusted for age and blood pressure.

Results

Repeatability for the measurement of carotid PWV was good (Cronbach's Alpha=0.703; Bland-Altman plot bias=0.25, upper and lower limits of agreement=+4.23, -3.74; n=16). Similarly, there was good intra-observer consistency (Cronbach's Alpha=0.747; Bland-Altman plot bias=0.06, upper and lower limits of agreement=+1.90, -1,78; n=13). The mean difference in transit time with and without static phantom correction for background velocity was 0.12 ms, P=0.4.

There was no significant difference in carotid PWV in healthy volunteers (median [IQR]: 4.1 m/s [2.8-5.60]) and patients with carotid artery stenosis (70-99%: 3.5 m/s [1.4-3.5], 50-69%: 4.9 m/s [2.5-5.3], <30%: 4.5 m/s [2.3-5.8]). There was also no association between carotid PWV and the degree of carotid stenosis (R^2=0.055, P=0.943). However, carotid PWV was significantly associated with increasing age group (R^2=0.35, P=0.001). None of the blood pressure parameters were significantly associated with carotid PWV (systolic blood pressure, R^2=0.047, P=0.07; diastolic blood pressure, R^2=0.007, P=0.5; pulse pressure, R^2=0.038, P=0.1; and mean arterial blood pressure, R^2=0.028, P=0.1).

There was a trend for higher PWV in carotids with MRIPH+ (4.14 m/s [1.9-4.7] versus 3.0 m/s [1.9-4.7], P=0.09), but it was insignificant. However, MRIPH+ carotid arteries were significantly associated with higher carotid PWV after adjusting for age, blood pressure and the degree of carotid stenosis (OR 1.22, 95% CI 1.02-1.47, P=0.03). This association was maintained even after exclusion of high grade carotid stenosis due to potential error in image acquisition in this group (OR 1.21, 95% CI 0.99-1.48, P=0.05). Furthermore,

symptomatic carotid stenosis was found to have higher value of carotid PWV compared to asymptomatic ones (OR 1.39, 95% CI 1.0-1.8, P=0.007).

Conclusion

This study has demonstrated the feasibility of assessing PWV in the carotid arteries with MRI. However, more work is needed to optimise the temporal and spatial resolution of the MRI sequence and to validate the technique. The association between MRIPH and carotid PWV requires further study with a larger cohort of symptomatic patients.

Acknowledgements

This pilot study on the feasibility of MRI pulse wave velocity could not have been made possible without the help and contribution from my esteemed colleagues.

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Contents

Abbreviations

AT	Applanation Tonometry
baPWV	Brachial-Ankle PWV
CEA	Carotid Endarterectomy
Cine-PC	Cine Phase Contrast
CCA	Common carotid artery
CI	Confidence Interval
cfPWV	Carotid-Femoral PWV
ICA	Internal carotid artery
ICH	Intracerebral Haemorrhage
IPH	Intraplaque Haemorrhage
MRIPH	MRI Detected Intraplaque Haemorrhage
MRIPH+	Presence of MRI Detected Intraplaque Haemorrhage
MRIPH-	Absence of MRI Detected Intraplaque Haemorrhage
MRI	Magnetic Resonance Imaging
NO	Nitric oxide
OR	Odds Ratio
PWV	Pulse Wave Velocity
ROI	Region of Interest

RR	Relative Risk
TIA	Transient Ischaemic Attack
ToF	Time of Flight
VSMC	Vascular smooth muscle cells

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ROI was first drawn around the hyperintense signal on the carotid plaque. A second ROI was drawn on the adjacent sternocleidomastoid muscle. The ratio of the intensity of the carotid plaque and the muscle was calculated (Icar / Imus). Carotid plaque was considered plaque haemorrhage positive if the ratio was 150% or more.

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Chapter 1

Introduction

1.1 Background

The contemporary management of symptomatic and asymptomatic extracranial carotid artery disease is mainly determined by the severity of intra-luminal stenosis based on ultrasound or arteriographic imaging. This was warranted by the results of several large trials such as the North American Symptomatic Carotid Endarterectomy Trial, the European Carotid Surgery Trial, the Asymptomatic Carotid Atherosclerosis Study and the Asymptomatic Carotid Surgery Trial (1-4). The benefit of CEA is maximised when surgery is performed rapidly following the precipitating neurological event as highlighted by Rothwell et al (5) who advocated that the time between surgery and patient's last symptoms should ideally be less than two weeks. However, these studies (1,6) also found that 70% of patients with a >70% intra-luminal stenosis remain asymptomatic over three years on best medical therapy alone. This indicates that the degree of stenosis does not have a high predictive value, although it is a valid marker for the risk of stroke. Over the last two decades, numerous studies have been published exploring better diagnostic tools in risk stratification of high grade carotid artery stenosis. Factors that have been taken into account include plaque composition, presence and state of the fibrous cap, intraplaque haemorrhage (IPH), plaque location, presence of ulceration, calcification and more recently, arterial stiffness (7).

Central arterial stiffness quantified by pulse wave velocity (PWV) has been shown to correlate with cardiovascular risk factors and predicts cardiovascular

events and all-cause mortality (8). The prognostic importance of PWV as a surrogate marker of arterial stiffness was first highlighted in renal patients where the probability of cardiovascular-free event is greatly reduced in patients with high PWV (9). Despite several studies that have been done on the correlation between central aortic stiffness and stroke, the results do not represent local arterial stiffness and cannot be used to identify high risk patients with high grade carotid stenosis. On the other hand, carotid stiffness is of interest as it may affect the likelihood of stroke by a variety of mechanisms. An increase in local arterial stiffness may result in a locally raised pulse pressure caused by increased systolic blood pressure and decreased diastolic blood pressure secondary to early wave reflection. This alters the haemodynamic and arterial remodelling of the extra-cranial carotid arteries, promoting carotid wall thickening, development of intra-luminal stenosis and ultimately plaque rupture (7). Furthermore, increased in central arterial stiffness has been shown to be associated with microvascular dysfunction (10,11). This may cause impairment of cerebral autoregulation, which reduces cerebral blood flow (12) that leads to silent cerebral infarct and declined cognitive function.

The scope of this thesis focuses on the concept and development of measuring carotid artery stiffness with PWV using MRI. It is a relatively new imaging technique that utilises the intrinsic motion sensitivity of MRI to quantify blood flow and motion of tissue. In this chapter, we shall first discuss the basic structure of arterial wall and the physiology of pulse wave. We shall then introduce the concept of PWV and the effects of mechanical and

haemodynamic forces that may have on it. We follow this by discussing the pathomechanism of arterial stiffness and the current consensus on quantifying PWV using non-invasive methods. To end the first chapter, we shall discuss the aims of this work and the research questions that we are hoping to answer. In the second chapter, we shall give an overview of the current literature on the association between arterial stiffness and stroke. We then describe in details the development of MRI PWV in the carotid arteries in the third chapter. In the last two chapters, we shall explore the differences of carotid PWV between patients and healthy volunteers as well as its association with other cardiovascular risk factors and MRI detected carotid IPH.

1.2 Structure of The Arterial Wall

To appreciate the concept of PWV and its implication on arterial stiffness, the composition of the arterial wall and theory of pulse pressure waveform should first be discussed. The main elastic materials of the arterial wall are collagen and elastin, with minor components such as fibrillin and glycoproteins associated with microfibrils. They are fibrous in nature, complexed with an amorphous substance formed from mucoprotein (13). The substance contributes to the elastic properties of the artery although it is not elastic by itself. The arterial wall can be divided in to three zones: tunica intima, tunica media and adventitia. The tunica intima consists of a single layer of endothelium and a thin layer of elastin and collagen that anchors it to the internal elastic lamina, which itself is a fenestrated membrane of elastin (13). The tunica intima is capable of detecting shear stress and is held together by a polysaccharide matrix, surrounded by a thin layer of connective tissue with the internal elastic lamina (14). The tunica media contributes to a large part of the arterial wall and is the main determinant of the mechanical properties of the arterial wall (13). It contains smooth muscle cells sandwiched between layers of fibrous structure consisting of organised elastin and collagen. This is followed by external elastic lamina, which demarcates the tunica adventitia. The adventitia is a layer of collagen with some elastin tissues that merge with the surrounding tissue (13). In larger blood vessels, the adventitia contains vasa vasorum and nerve fibers that supply the vessel (14).

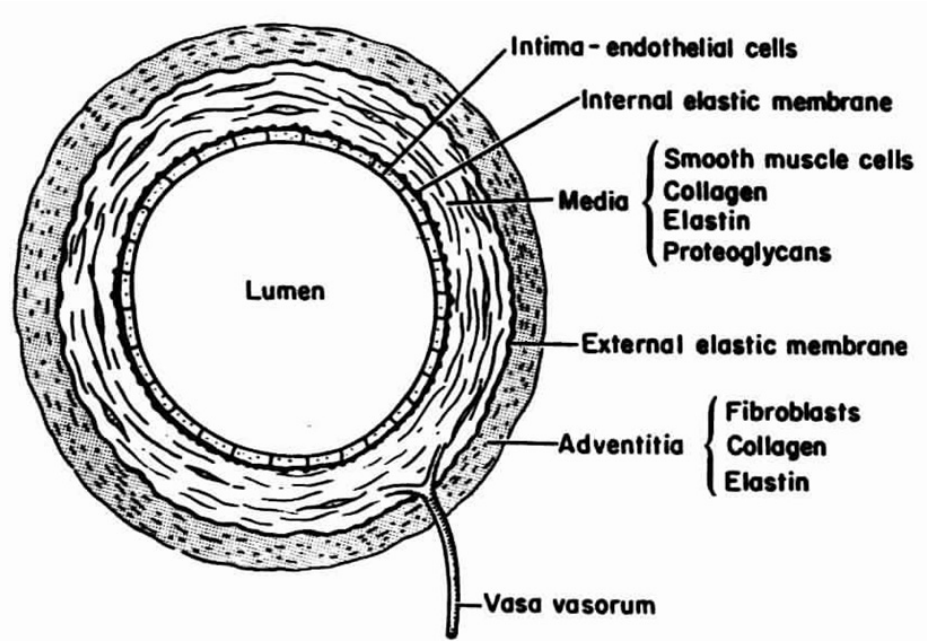


Figure 1.1: Cross-sectional view of the layers of arterial wall.

The distribution of collagen and elastin differs remarkably between central and peripheral arteries. In the thoracic aorta, the fibrous element of the arterial wall consists of 60% of elastin and 40% of collagen (13). There is approximately five centimetres of transitional zone in the distal thoracic aorta before piercing the diaphragm and at the branches leaving the aortic arch. From there the composition of fibrous tissue changes to 30% of elastin and 70% of collagen in the distal branches (13). The elastic modulus of collagen is much higher than that of elastin. As the distance from the heart increases, the arteries become stiffer and the elastic modulus as well as PWV increases. This relationship is demonstrated by the Moens-Korteweg equation, which will be discussed in the next section.

1.3 The Moens-Korteweg Equation

$$PWV = \sqrt{\frac{Eh}{2r\rho}}$$

Figure 1.2: The Moen-Korteweg equation: E = incremental elastic modulus of the vessel wall, h = wall thickness, r = vessel radius, and ρ = blood density. It is assumed that the arterial wall is isotropic and experiences isovolumetric change with pulse pressure.

As discussed previously, the arteries become stiffer and the elastic modulus as well as PWV increases as the distance from the heart increases. The relationship between distance and stiffness can be explained by the Moens-Korteweg equation, we shall discuss only the basic principles here.

The Moens-Korteweg equation is a biomechanical model that describes the relationship between wave speed and the incremental elastic modulus of the arterial wall or its distensibility. As the name of the equation implies, it was derived from Newton's second law of motion by Adriaan Isebree Moens and Diederik Kortweg, a Dutch physician and a mathematician. They derived this mathematical formula by studying the velocity of a pressure wave in a rubber

tube filled with water (13). The equation has made two assumptions. Firstly, the rubber tube has a thin wall and secondly, it is filled with an ideal incompressible inviscid liquid (13). In large arteries, this model is reasonable as the effect of viscosity is small. However, this will significantly retard the flow velocity in smaller arteries assuming the arterial wall is thin (13). Bergel later explored the equation and incorporated Poisson's ratio to correct the assumption. It was reported that the difference in results between Moen-Korteweg and the corrected equation by Bergel was found between 16% and 24% (13). This discrepancy was considered to be negligible as there was considerable measurement error in PWV at the time. Later on, Nichols and McDonald were able to reduce the standard error of measurements to within three percent (13). Several methods have since been developed by others to measure arterial stiffness and PWV but have not been proven to be more accurate.

With the understanding in the composition and variability of elasticity of the arterial wall in mind, we shall discuss the pulse waveform and how arterial stiffness can augment the contour of the waveform.

1.4 The Pulse Waveform

The pulse is a series of oscillations traveling along the arterial vasculature and can be described in the forms of pressure, flow and dimension (15). When blood is ejected from the left ventricle, a pulse wave (incident wave) is generated to propel blood in to the aorta and the peripheries. The pulse wave passes through bifurcations and arterioles where there is a change in caliber and increasing resistance (impedance), which act as a mirror reflecting the wave back to the aorta (reflected wave). The reflected wave meets the forwarding incident wave and causes an augmentation of pulse pressure waveform resulting in raised diastolic blood pressure in a healthy elastic aorta. In a stiffened aorta, the increase in PWV causes premature return of the reflected wave in late systole resulting in a higher systolic blood pressure and a drop in diastolic blood pressure, hence a higher pulse pressure. Moreover, a stiffer arterial wall disrupts the Windkessel effect in large-sized arteries resulting in cyclic stress associated with increased systolic pressure and reduction in coronary perfusion (16). The aorta not merely acts as a conduit for blood flow but also as a central elastic reservoir to dampen excessive pressure pulsation in stiffer peripheral arteries. While a portion of energy from cardiac systole generates pulse wave to propel blood forward, the remainder is stored as potential energy in the distended aorta (16). During diastole, the stored energy is being utilised to perfuse the coronary and peripheral arteries. The protective effect of the arterial wall against the pulsatility of blood pressure decreases with age. The question of whether arterial stiffness itself

is a risk factor or a consequence of atherosclerosis remains debatable. Nonetheless, studies have suggested several mechanical and haemodynamic factors that may influence the development of atherosclerosis.

1.5 Mechanical Forces On The Arterial Wall

Atherosclerosis is an established risk factor for acute ischaemic stroke. Its importance has been highlighted decades ago by several studies including the Framingham study where high incidence of embolic stroke of non-cardiac origin was emphasised (17). Later, the term 'vulnerable plaque' was adopted to describe plaques at high risk of thrombosis and high probability of undergoing rapid progression to become culprit plaques (18). Vulnerable plaques that are at high risk of rupture often have a large necrotic core covered by a thin fibrous cap (19,20). It may contain IPH, calcification and inflammation (19,20). However, there is a poor correlation between the degree of carotid artery stenosis and the risk of carotid plaque rupture (21). This was supported by histological studies that suggested an absence of association between symptoms and complicated plaques (22,23). Mechanical factors that have been suggested to potentially affect the arterial wall include blood shear stress, pressure forces and structural forces. Many of the studies on mechanical properties of the atherosclerotic plaque have been performed with computational model based on in vivo plaque imaging using finite element analysis (FEA) and fluid structure interaction (FSI) (24-27).

The rate and magnitude of change in blood flow on the intra-luminal surface have been associated with the pathogenesis of atherosclerosis. A complex pattern of fluid velocities occurs when blood flow separates at an arterial bifurcation. Flow separation occurs when the highest velocities at the center of flow come in to contact with the flow divider and the flow separation occurs

until some distance in to the distal branches (28). The blood flow near the centre of the artery remains laminar and has a higher shear stress than blood flow at the corner, which is often slower with disturbed currents (28). This area often has a lower shear stress and has higher incidence of developing atherosclerotic plaque (28). Li et al (29) evaluated whether wall shear stress and pressure gradient may cause plaque rupture. They found that pressure gradient was the main trigger for plaque rupture and normal to high shear stress are atheroprotective. Low wall shear stress plays an important role in attenuating nitric oxide-dependent atheroprotection and promotes lower density lipoprotein cholesterol uptake, synthesis and permeability (29). Furthermore, studies have found increasing severity of stenosis decreases stress, which support the above findings (25,30).

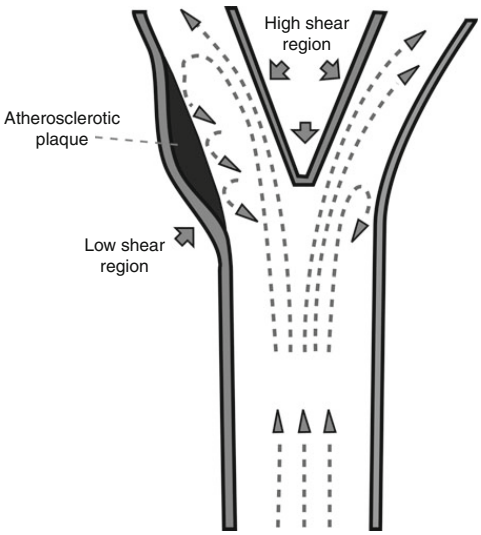


Figure 1.3: Wall shear stress with boundary layer separation. The pathomechanism of plaque formation with low shear stress is a complex process that include modulation of endothelial gene expression resulting in reduced synthesis of endothelial NO. This promotes increase uptake of low density lipoprotein cholesterol, inflammation and vascular smooth muscle cell proliferation (28).

Several studies have examined other aspects of mechanical properties of the atherosclerotic plaque. Takano et al (31) investigated coronary artery plaque using coronary angioscopy and classified them in to yellow and white plaques. Yellow plaques have thin fibrous cap with lipid-rich cores and inadequate collagen content whereas white plaques have thick fibrous caps or are completely fibrous. They found that yellow plaques have an increased distensibility with a compensatory enlargement and may be mechanically as well as structurally weak. Huang et al (24) examined the correlation between carotid IPH and structural stress of the plaque using FSI model. Their results

showed that higher plaque wall stress has a better correlation with carotid IPH compared to flow shear stress. Using the same technique, Tang et al (25) found that plaque cap weakening may lead to larger strain increase but minimal change in stress level under pulsating pressure and more severe stenosis may lead to lower stress levels. Huang et al (32) studied fibrous cap fatigue secondary to arterial pulsatility during each cardiac cycle using MRI. They found that the fatigue cracks initially occurred at the vulnerable sites had consequently developed fibrous cap rupture. Furthermore, once the fatigue crack had occurred, it increases at an exponential rate during further loading cycle (32). The material strength of an intact fibrous cap of carotid plaque is about 1000 kPa while the critical mechanical stress in the ruptured plaque is about 500 kPa (33-35). Hence, peaks in systolic or diastolic blood pressure alone may not be sufficient to cause plaque rupture but the insidious accumulation of injury induced by cyclic stresses could be a cause (32).

In the following chapter, the pathomechanism of arterial stiffness and the changes in its composition are discussed.

1.6 Pathomechanism of Arterial Stiffness

The pathomechanism of arterial stiffness is multi-factorial. The topic for debate remains whether atherosclerosis results in increased arterial stiffness or vice versa. Some argued that increased arterial stiffness could be considered as an intermediate factor in the pathway between risk factors and cardiovascular disease (36,37). This is because arterial stiffness is a risk factor for cardiovascular disease in certain populations, but it is also the result of detrimental effect on the arterial wall by other cardiovascular risk factors (36). In spite of that, it is well established that age and hypertension are the *sine qua nons* of arterial stiffness and the pathomechanism of both may overlap each other. The two main risk factors (age and blood pressure) of arterial stiffness are discussed here.

1.6.1 Age

The process of arterial stiffness secondary to ageing can generally be categorised in to three broad areas: extracellular matrix, endothelial dysfunction and vascular smooth muscle cells.

I. Extracellular Matrix

With ageing, the absolute amount of elastin and collagen fall (with decrease in collagen to a lesser extent) due to an increase in fat content and extracellular material, for example calcium (38). The increase in arterial stiffness with age is progressive from childhood and is characterised by fracture and depletion of elastic load-bearing elements as well as collagen deposition (13). This causes the wall to weaken and to stretch so that stress is transferred to collagenous elements in the wall (13). In vitro study using human arteries demonstrated that fragmenting elastin using elastase in young artery shifts the incremental elastic modulus exponentially to the right (39). This produces a collagen-dominated curve which is similar to the shape in those of old control arteries (39). Furthermore, there is migration and proliferation of medial vascular smooth muscle cells causing the intima of the conduit arteries to thicken (40). In addition, there is progressive increase in luminal diameter and lengthening of vessels, resulting in tortuosity (41,42). It has been suggested that the thinning and fragmentation of the arterial elastin fibres in ageing is secondary to fatigue failure (43). However, the current evidence in

the literature is limited to support this theory (44). In addition, there is evidence to suggest that the stiffening of the remaining elastic tissue is due to chemical degradation and calcification. A more diffuse accretion of calcium salts called medial elastocalcinosis is seen in the aortic media where it is often linked with arteriosclerosis in the elderly and patients with diabetes mellitus (44-46). This was supported by post-mortem study that showed the prevalence of aortic calcification increases with age (47). Although this only affects 4% of the population during their third decade of life but almost all were affected by the age of 50 (47).

II. Endothelial Dysfunction

The endothelium is a single-cell layer that lines the innermost surface of blood vessels. Its importance was first recognised by its effect on vascular tone. This was found by the secretion of several vasoactive molecules that relax or constrict the vessel, and also by response to and modification of circulating vasoactive mediators such as bradykinin and thrombin (48). One of the most important substance that maintains the integrity of vascular endothelium is nitric oxide (NO) (49). NO is an endothelium-derived relaxing factor and its importance was first demonstrated by the pioneering experiments of Furchgott and Zawadzki (50). It has a key role in the maintenance of vascular wall in quiescent state by inhibition of platelet and white cell activation, maintain the vascular smooth muscle cells (VSMC) in a non-proliferative state and prevent

thrombosis (48). NO is generated from L-arginine by the action of endothelial NO synthase (eNOS) together with co-factors such as tetrahydrobiopterin (51). Study has shown that shear stress was the key factor in the activation of eNOS and this causes organ perfusion to adapt to changes in cardiac output. Furthermore, eNOS may also be activated by others such as bradykinin, adenosine, vascular endothelial growth factor and serotonin (52). Moreover, the endothelium also mediates hyperpolarisation of VSMC via a NO independent pathway. This increases potassium conductance and subsequent propagation of depolarisation of VSMC to maintain vasodilatory tone. Besides vasodilatory substances, endothelium also modulates vascular tone through production of endothelin and vasoconstrictor prostanoids, as well as conversion of angiotensin I to angiotensin II at the endothelial surface (53,54). These vasoconstriction agents mainly act locally but also have some systemic effects and the regulation of arterial structure and remodelling (48). It has been shown that laminar shear stress may be the major factor that maintains the quiescent state of NO dominated endothelial phenotype (55).

In the presence of endothelial injury such as those secondary to cardiovascular risk factors, activation of endothelium occurs. Endothelial activation represents an activation from a quiescent phenotype to one that incorporates the host defence response (48). The change in fundamental process involved is an alteration in signalling from a NO mediated silencing of cellular processes to activation by redox signalling (48). This results in switching of eNOS from maintaining the quiescent state of the endothelium to generation of reactive oxygen species (ROS) in appropriate circumstances as

part of endothelial activation. ROS are highly reactive molecules that originate mainly from the mitochondrial electron transport chain (56). They play a key role in cell signalling and homeostasis.

Hypertension is one of the commonest diseases in the elderly. The prevalence of hypertension markedly increases with advancing age (57). In such circumstances, chronic shear stress on the endothelium leads to chronic production of ROS. This may exceed the capacity of cellular enzymatic and non-enzymatic anti-oxidants and therefore induction of sustained endothelial activation. This will ultimately exhaust the protective effect of endogenous anti-inflammatory system within endothelial cells, leading to endothelial dysfunction. This is characterised by loss of cell integrity, senescence and eventually detachment in to the circulation (58). Although repair occurs by replication of adjacent mature endothelial cells and circulating endothelial progenitor cells originating from bone marrow (59), the latter is NO dependent and may be impaired in the presence of cardiovascular risk factors (60). This ultimately leads to reduction in NO and hence impaired inhibition to activation of inflammatory process and switching of VSMC from contractile state to synthetic state. The latter will be discussed in the next section.

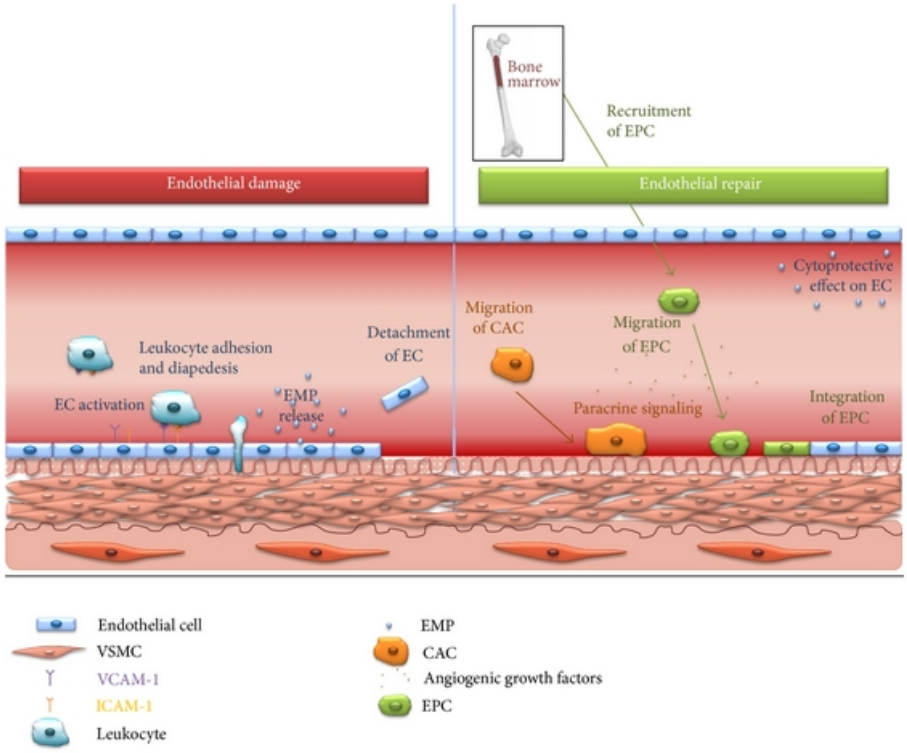


Figure 1.4: Endothelial injury and repair (61).

III. Vascular Smooth Muscle Cells

Arterial stiffness in ageing has always been assumed to be secondary to changes in the extracellular matrix (45,62,63) and endothelium (64-66). Recent studies have found novel mechanism for vascular stiffness in ageing that involved the intrinsic mechanical properties of VSMC (67,68). VSMC exist in several phenotypes: contractile, synthetic, migratory and proliferative (69,70). In normal physiology, the predominant VSMC are in quiescent state and exhibit contractile phenotype. However, during environmental stress, VSMC can switch to one of the other three phenotypes, depending on the response to injury. During pathogenic vascular remodelling, VSMC with non-

contractile or synthetic (termed dedifferentiated cells) phenotype generate intimal vascular lesions (71,72) and have increased capacity to generate extracellular matrix protein (69,70). This include collagen, elastin, proteoglycans, growth factors and cytokines. In addition, dysregulation of VSMC function is exacerbated by impairment in sympathetic nervous system function (73).

A recent study using animal model showed that the total elastic modulus of the VSMC in the thoracic aorta of old male monkeys was significantly higher than those in the young (67). Further assessment of the mechanical properties of VSMC using atomic force microscopy and reconstituted tissue model showed both beta1-integrin and alpha-smooth muscle actin are likely major players in the increased stiffness of VSMC during ageing (67). As previously discussed, ageing is closely related to hypertension and both have an overlapping role in the pathomechanism of arterial stiffness. Another study using animal model suggested that arterial stiffness is affected by inhibitors of proteins related to contractile function in VSMC (e.g. actin, myosin light chain kinase) (68). This was further supported by the increased expression of these proteins that was found in spontaneously hypertensive rats as compared to control (68). This suggested that contractile and cytoskeletal proteins play an important role in determining VSMC stiffness (68).

1.6.2 Blood Pressure

The main functions of the artery are two-folds: to act as a conduit to blood flow and to sustain blood pressure (74). The frictional force of the blood (wall shear stress) on the endothelium is opposed by tension and deformation in the endothelium while the circumferential distension of blood pressure is opposed by circumferential stress and strain in the vessel wall (75). In addition, pulsatile flow secondary to the cardiac cycle creates an oscillatory shear stress (28). Blood flow near the center of the artery is laminar whereas those near the intima is known as the boundary layer. The latter is slower and has more turbulent currents and this is known as boundary layer separation. The areas of lower shear force have been found to initiate endothelial injury and are typically found on the outer walls at arterial branch points as demonstrated in the carotid bifurcation (28). The details of these have been discussed in Chapter 1.5.

A perturbation of the dynamic balance between mechanical or chemical stimulus and biological response can lead to physiological adaptation or disease of the vessel wall. It is well documented that atherosclerosis develops in regions subjected to elevated blood pressure (75). This is evident by the absence of atherosclerosis in veins except when it is subjected to arterial pressure (75,76). Another example is the pulmonary arteries where atherosclerosis develops only in pulmonary hypertension (77). Moreover, lower limbs are more likely to develop atherosclerosis than upper limbs as the former has a higher pressure due to hydrostatic pressure (74,78). Studies

have also suggested that increased heart rate may promote arterial stiffness by exposing the vessel wall to a greater cyclical stress (79,80). Nonetheless, this is inconsistent as other studies disagree and proposed that increased heart rate may be a confounding factor for arterial stiffness as it is a variation in blood pressure (81,82). The mechanobiology of arterial wall response to stress is complex. It involves activation of various chemical mediators and signalling pathways in the endothelium as well as the extracellular matrix, which eventually lead to compensatory vascular remodelling.

Aortic PWV has been shown to increase exponentially with ageing and therefore it is a sensitive indicator of physiologic stiffness after the age of 50 to 60 years (83). A cohort study with six years of follow-up demonstrated that normotensive subjects of less than 50 years of age have no evidence of aortic stiffness (84). However, in the presence of hypertension, there was an accelerated progression of aortic stiffness in the same age group of subjects (84). Although blood pressure is one of the strongest factors influencing PWV, varying correlation coefficients have been reported between the various PWV (carotid-femoral, brachial-ankle, etc) and blood pressure parameters (85,86). Several studies have reported that aortic PWV was correlated with systolic blood pressure and mean arterial pressure but not with diastolic blood pressure (86,87). However, some studies found that aortic PWV was correlated with systolic blood pressure only (88,89) or systolic and diastolic blood pressure only (90). Furthermore, pulse pressure was shown to be the strongest correlation with aortic PWV among other blood pressure parameters in normotensive and untreated hypertensive elderly subjects using invasive

blood pressure measurements (91). In contrary, Nurnberger et al (92) had suggested that diastolic blood pressure was the only determinant of aortic PWV among all blood pressure parameters in young healthy male. The findings of this study reinforced the evidence of age related different relation between blood pressure parameters and PWV (91). The mixed results observed in these studies could be explained by the inevitable variability of both PWV and blood pressure within individual subjects, the heterogeneity of study design, the demographics of study population as well as the method of PWV measurement. In addition, the possibility of overestimation of blood pressure in the presence of severe mediacalcosis due to the lack of compressibility of peripheral arteries should be taken in to account (93).

1.6.3 Others

Several other factors have been associated with arterial stiffness. Matrix metalloproteinases are a family of proteolytic enzymes that degrade various components of extracellular matrix by affecting the production of weaker collagen and frayed elastin fibers (94). Recent study has demonstrated that arterial stiffness is correlated specifically with serum metalloproteinases-2 and metalloproteinases-9 levels as well as serum elastase activity in hypertensive adult and normotensive young individuals (95). The accumulation of advanced glycation end products has been associated with cross-linking of collagen that leads to increased arterial stiffness (44). Hyperglycaemic conditions enhance the cross-link formation and hence arterial stiffness is more severe in diabetes mellitus (44). The production of vascular endothelial NO decreases with age. This results in an increase in resting vascular smooth muscle cells tone due to reduced availability of NO, leading to increased mean blood pressure and greater stretch of conduit arteries (44). Lastly, it has been suggested that arterial stiffness may have a genetic component which is largely independent of the influence of blood pressure, heart rate, age and other cardiovascular risk factors (96).

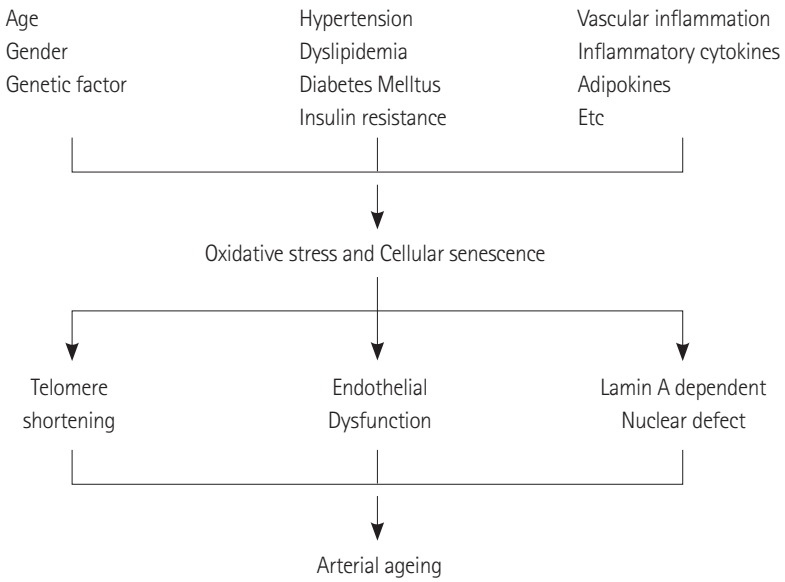


Figure 1.5: Pathomechanism of arterial stiffness in ageing (97).

1.7 Pulse Wave Velocity

1.7.1 Background

The study of PWV was first pioneered by Thomas Young as he derived a formula to calculate the relationship between PWV and arterial wall stiffness, which was later adopted in to the Moen-Korteweg equation (13). His work was expanded by others with description of the first quantitative sphygmograph (13). Unfortunately, studies on arterial stiffness lapsed after clinical acceptance of the sphygmomanometer. Since then, blood pressure had been the focus of research, culminating in large trials such as the Framingham study. Despite this, measurement of blood pressure from the brachial artery remains open to criticism; the left ventricle is likely to be influenced directly, not by the pressure in the brachial artery, but by the pressure in the ascending aorta (15,98,99). Over the last decade, the advances in imaging has allowed the rediscovery of PWV. Aortic stiffness has been shown to be an independent predictor for all cause and cardiovascular mortalities, fatal and non-fatal coronary events as well as fatal strokes in patients with uncomplicated essential hypertension, type 2 diabetes and end stage renal disease (7). In 2007, owing to substantial published evidence on PWV, the European Society of Hypertension produced a new guideline with the incorporation of aortic PWV as a recommended investigation for arterial hypertension. The predictive value of PWV could potentially be a new marker in the assessment and risk

stratification of cardiovascular and cerebrovascular disease. PWV can be measured using several methods and is discussed in the next section.

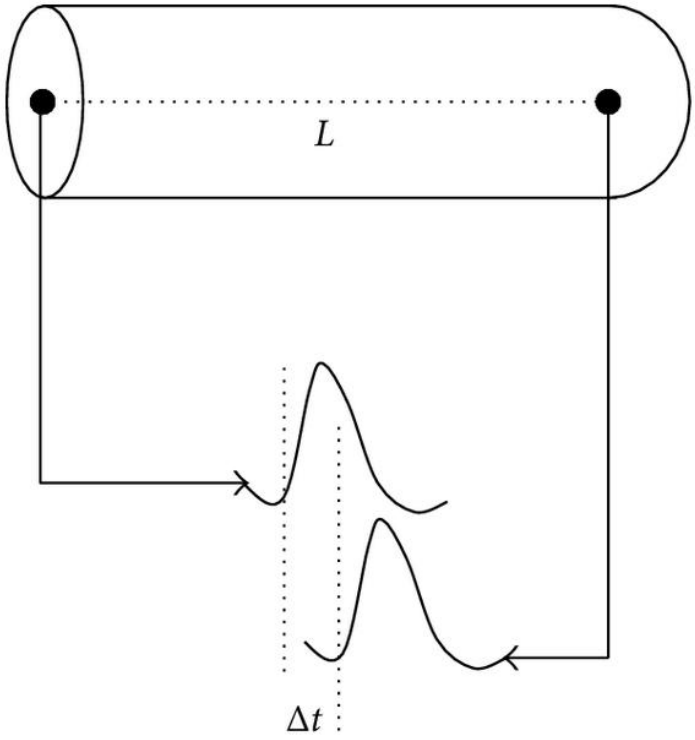
1.7.2 Measuring Pulse Wave Velocity

Although there are several methods of quantifying arterial stiffness, none is regarded as the gold standard (100,101). The measurement of PWV is generally accepted as the most simple, non-invasive, robust and reproducible method to determine arterial stiffness (7,100). It is defined as the speed of pulse wave traveling through the arterial tree. PWV is recorded by calculating the distance between two points on the arterial tree divided by the transit time, which is calculated from the foot of the pressure wave at the first point to the foot of the pressure wave as it arrives at the next point (15). However, the precision of this method depends highly on the exact calculation of flow difference and distance between only two measuring points (102). There are several non-invasive methods of recording PWV: applanation tonometry (AT), plethysmography, and recently MRI.

To date most studies on arterial stiffness and its associated target organ damage have been focused on the aorta. Brachial-ankle PWV (baPWV) and carotid-femoral PWV (cfPWV) are both valid methods for assessing aortic stiffness. The latter has been considered as the gold standard in the western countries (7). Both methods have a short acquisition time, are highly reproducible and can be performed as a bedside assessment.

The carotid artery is thought to be more in line with the aorta and the left ventricle, therefore demonstrating a stronger correlation in pressure change (7). The greater distance from the heart and the muscular nature of the brachial artery causes amplification phenomenon, thus may overestimate the value of PWV especially in younger subjects (7). Nonetheless, Japanese researchers advocate the use of baPWV and showed that aortic PWV was the primary independent correlate of it (7,103). Supporting this, Yu et al (104) found that baPWV correlates better with left ventricular mass and diastolic function and other indices of arterial function, probably because it encompasses a greater territory of arterial tree than cfPWV. Although both are valid ways of measuring aortic stiffness, some limitations should be considered. The femoral pressure waveform could be a challenge to be recorded accurately in patients with metabolic syndrome, peripheral artery disease, diabetes mellitus and obesity (105). The pressure wave may be attenuated and delayed In the existence of aortic, iliac or proximal femoral stenosis (106). Additionally, abdominal obesity, especially in men and women with large bust size can lead to inaccurate distance measurement (7). Joly et al (107) found that the cfPWV obtained by AT may not truly reflect the aortic PWV in subjects with body mass index of ≥35kgm^{-2}. Surface measurement of path length is also influenced moderately by age due to increasing arterial tortuosity and this can underestimate the absolute value of PWV (108). In contrast, PWV measured using MRI is more precise as the absolute distance travelled by the pulse wave can be accurately determined. It also allows PWV to be quantified in any part of the arterial tree. However, its accessibility can be a limiting factor. The majority of the studies on aortic PWV using MRI have

utilised cine phase contrast as the MRI sequence to quantify velocity and flow in the artery. In the next section, we shall discuss briefly the basic principles of this sequence.



$$\mathrm{PWV\ (m/s)} = L/\Delta t$$

Figure 1.6: PWV can be calculated by dividing the length (L) with time (t) obtained between foot to foot of velocity-time curve (109).

1.8 Cine Phase Contrast In Magnetic Resonance Imaging

Cine-PC is a MRI sequence that uses a combination of phase contrast and cine MRI to acquire both functional and morphological data within a single session. The image acquisition is often gated to the cardiac cycle and time resolved anatomical images are gathered to demonstrate tissue motion dynamics and blood flow during a cardiac cycle. Phase contrast utilises the basis on spins that move through magnetic field gradients obtain a different phase than static spins and therefore allowing production of images with controlled sensitivity to flow (110). On the other hand, Cine MRI utilises either a standard gradient echo pulse sequence, a segmented data acquisition, a gradient echo EPI sequence or a gradient echo with balanced gradient waveform. It acquires images by covering one full period of cardiac cycle or over several periods to obtain a complete coverage of a cardiac cycle (110).

The velocity encoding in Cine-PC is usually performed using bipolar gradients where moving spins will have a linear velocity dependant phase change, proportional to the amplitude and timing of the gradient. This results in the production of a modulus and phase image. A maximal velocity encoding has to be defined to avoid phase wrapping or phase aliasing.

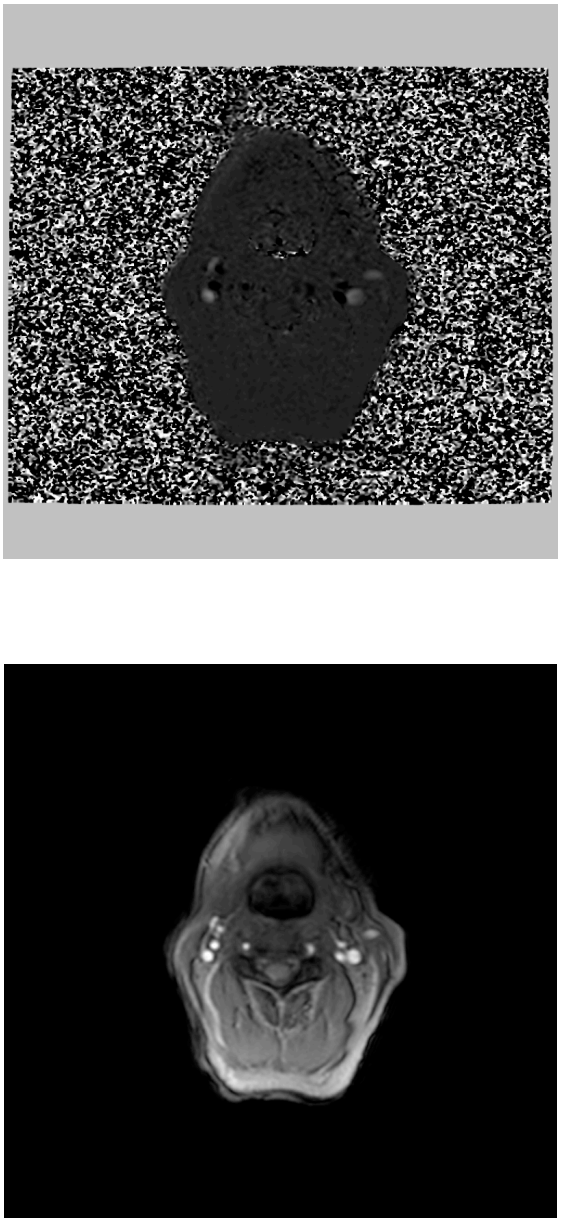


Figure 1.7: An example of a phase (top) and modulus (bottom) image of Cine-PC acquired at the carotid arteries.

1.9 Summary

Arterial stiffness has been studied for several decades now. Nevertheless, it has only been rediscovered recently, evident by the surge in studies on its pathomechanism and clinical implications. With the rapid development of technology, PWV can be measured non-invasively and has been shown to be a surrogate marker of arterial stiffness. Indeed many of the work published in the literature have focused on aortic PWV and its association with cardiovascular and cerebrovascular diseases. Recently, MRI has been shown to be feasible to measure PWV over a segment of an artery and this has led to an interest in assessing local arterial stiffness and its association with target organ injury.

The study of local arterial stiffness has been focused on carotid arteries. This is probably because of its close relationship with the brain and its relatively superficial anatomical location. The current evidence have shown the feasibility of assessing carotid PWV using MRI in healthy volunteers only. It is unknown if this is feasible in patients with carotid artery disease due to the arterial jet flow through intra-luminal stenosis. it is also uncertain whether carotid stiffness is higher in patients with carotid artery stenosis and whether it has a role in the development of vulnerable carotid plaque. Hence, this work aims to explore these areas in the form of pilot study.

1.10 Research Questions

1. What are the differences in carotid artery stiffness measured by MRI PWV in patients with moderate and high grade carotid artery stenosis compared to those in healthy volunteers?

2. Can carotid artery stiffness measured by MRI PWV predict the unstable carotid plaque quantified by MRIPH?

Chapter 2

Literature Review

2.1 Introduction

A literature review was performed to summarise the current evidence on arterial stiffness and stroke. The literature review was written in the structure of a systematic review.

2.2 Literature Search Strategy

A systematic literature review was undertaken for studies published between 1960 and January 2013 on the association between aortic and carotid arterial stiffness and cerebrovascular disease. An electronic search on MEDLINE and EMBASE was performed using pre-specified keywords: ‘stroke and pulse wave velocity’, ‘stroke and arterial stiffness’, ‘cerebrovascular disease pulse wave velocity’ and ‘cerebrovascular disease arterial stiffness’. Further search was performed by adding the word ‘carotid’ to all keywords above for articles published on the association between carotid artery stiffness and stroke. The electronic search was supplemented by a hand search of the reference list of each selected article.

All articles published on the association between arterial stiffness and cerebrovascular disease were included. Selection of articles were not restricted to English language only. Articles published on the association between arterial stiffness and atherosclerosis, coronary artery disease and peripheral arterial disease were excluded.

Arterial stiffness is defined as stiffness of the artery assessed by means of PWV using applanation tonometry (AT), plethysmography or MRI, or arterial distensibility using ultrasound or echocardiography. Cerebrovascular disease is defined as ischaemic or haemorrhagic stroke, transient ischaemic attack or silent cerebral infarction.

2.3 Aortic Stiffness and Stroke

13 articles (3 cohort (80,111,112), 6 cross-sectional (10,113-117), 3 case-controlled (36,118,119) and 1 cohort and cross-sectional (120)) reported on the association between aortic stiffness and stroke were identified between 2001 and 2011 (Table 2.1). Hatanaka et al (115) reported the objective and hypothesis of the study. Several other authors (10,36,80,111,112,114,118-120) reported only the objective(s) of their study whereas Nakano et al (116) and Nomura et al (117) did not specify either the objective or the hypothesis of their study.

2.3.1 Outcome Measures

In both cohort and cross-sectional design, five studies (80,111,112,116,120) investigated the association of aortic stiffness with cerebrovascular disease (both haemorrhagic and ischaemic stroke). One study (114) investigated the association between aortic stiffness and intracranial large artery disease. Two studies (114,115) on silent cerebrovascular lesion and two studies (10,113) on lacunar infarct. In the case-controlled studies, in addition to PWV, risk factors of ischaemic stroke (118), cardiovascular risk factors (119) and laboratory as well as clinical variables (36) were investigated.

2.3.2 Characteristics of Study Population

Henskens et al (10), Laurent et al (111) and Brandts et al (113) recruited patients from the hypertension outpatient clinic. Nomura et al (117) recruited patients with type 2 diabetes mellitus who have attended hospital. Nakano et al (120) recruited elderly patients who have been treated in the geriatric department. De Silva et al (114), Yokokawa et al (119) and Tuttolomondo et al (36) recruited patients who were admitted to hospital with acute ischaemic stroke. Sugioka et al (118) recruited patients with ischaemic stroke who were referred for transesophageal echocardiography. However, it was not clear whether these were all inpatients.

Mattace-Raso et al (112) and Hatanaka et al (115) performed their study on community based, well-functioning subjects. Sutton-Tyrrell et al (80) recruited random community based subjects who were Medicare beneficiaries. Nakano et al (120) did not provide details on patient recruitment. All except three authors (111,116,120) reported the eligibility criteria of their studies.

Age is important in the study of PWV as it is a confounding factor. Brandts et al (113) recruited patients below the age of 50. Henskens et al (10), Nakano et al (116) and Laurent et al (111) recruited patients with mean age between 50 to 60. Nomura et al (117), Hatanaka et al (115), Nakano et al (120) and Sugioka et al (118) recruited patients with mean age between 60 to 70 years. De Silva et al (114) recruited patients with median age of 60 to 65. The other authors (36,80,112) recruited patients with mean age above 70. Yokokawa et

al (119) did not report on the age group of the study population.

2.3.3 Assessment of Aortic Stiffness

Seven studies (10,36,80,111,112,114,120) measured aortic stiffness with cfPWV and four studies (115-117,119) with baPWV using either AT or plethysmography. Two other studies measured aortic arch PWV using MRI (113) and transesophageal echocardiography (118). Aortic PWV measured with AT, plethysmography and MRI have been validated previously (121,122).

2.3.4 Association between Aortic Stiffness and Stroke

In cohort studies, Laurent et al (111) showed that hypertensive patient with higher cfPWV is more likely to have fatal stroke (RR 1.39, 95% CI 1.08-1.72, P=0.02) following adjustment for cardiovascular confounders. Sutton-Tyrrell et al (80) and Mattace-Raso et al (112) found similar results in well-functioning individuals with higher cfPWV. However, the latter was not statistically significant (RR 2.6, 95% CI 1.19-5.64, P=<0.05; HR 1.96, 95% CI 0.94-4.29, P=0.06). In the cohort section of the study, Nakano et al (120) showed a positive correlation between cfPWV of ≥10 meter/seconds and stroke with an extremely wide range of CI (RR 20.2, 95% CI 2-203, P=0.011).

On multiple logistic regression, Nakano et al (116) demonstrated that baPWV

was weakly associated with ICH (OR 1.003, 95% CI 1.000-1.005, P=0.0243) and cerebral infarction (OR 1.003, 95% CI 1.001-1.004, P=<0.001). Henskens et al (10) and Brandts et al (113) have demonstrated that cfPWV and aortic arch PWV in patients with hypertension was associated with lacunar infarction (OR 1.78; 95% CI: 1.06-2.99, P=0.05; OR 1.8, 95% CI 1-3, P=0.04). De Silva et al (114) demonstrated that patients with intracranial large artery disease were significantly more likely to have cfPWV in the highest quartile (OR 2.21, P=0.038). On the other hand, Nomura et al (117) showed that baPWV in patients with type 2 diabetes mellitus was not an independent predictor of silent cerebral infarct (OR 1.0, 95% CI 1-1, P=0.617). In contrast with Nomura et al (117), Hatanaka et al (115) found a statistically insignificant positive correlation between baPWV and silent cerebral infarct in well-functioning individuals (OR 1.82, 95% CI 0.94-3.55, P=0.07). Similarly, in the cross-sectional section of the study, Nakano et al (120) found the association between cfPWV and stroke was not statistically significant (OR 1.49, 95% CI 0.76-2.90, P=0.243).

In case-controlled studies, Sugioka et al (118) demonstrated that aortic arch stiffness as measured by distensibility was independently associated with ischaemic stroke (OR 1.28, 95% CI 1.11-1.52). However, the precision of he result was not reported. Yokokawa et al (119) and Tuttolomondo et al (36) showed that baPWV (OR 2.92, 95% CI 1.0-8.51, P=<0.05) and cfPWV (P=<0.001) were higher in patients with acute ischaemic stroke after adjusted for confounders. Nevertheless, the latter calculated the Pearson's correlation coefficients but not the effect size and CI of the association.

First author, year	Design	Mean age	Assessment of arterial stiffness	Characteristic of patients	Outcome measure(s)
Nakano, 2001	Coh and CS	68±11	cfPWV	Well-functioning	IS and TIA
Sugioka, 2002	CC	69±9	Aortic arch distensibility	Ischaemic stroke	Acute IS
Laurent, 2003	Coh	51±13	cfPWV	Hypertension	All strokes
Nakano, 2004	CC	56-60	baPWV	All strokes	IS, ICH and SAH
Sutton-Tyrrell, 2005	Coh	74±3	cfPWV	Well-functioning	All strokes
Mattace-Raso, 2006	Coh	72±7	cfPWV	Well-functioning	All strokes
Yokokawa, 2007	CC	NA	baPWV	Ischaemic stroke	IS
Henskens, 2008	CS	31-69	cfPWV	Hypertension	Lacunar infarction
De Silva, 2009	CS	61-63*	cfPWV	Well-functioning	ICLAD
Brandts, 2009	CS	49±13	MRI aortic arch	Hypertension	Lacunar infarction
Nomura, 2010	CS	67±9	baPWV	Type 2 DM	Silent cerebral infarction
Tuttolomondo, 2010	CC	71	cfPWV	Ischaemic stroke	Acute IS
Hatanaka, 2011	CS	66±6	baPWV	Well-functioning	Lacunar infarction

Coh = Cohort, CC = Case-controlled, CS = Cross-sectional, NA = Not available, * = Median, cfPWV = carotid-femoral PWV, baPWV = brachial-ankle PWV, MRI = Magnetic resonance imaging, DM = diabetes mellitus, IS = ischaemic stroke, TIA = transient ischaemic attack, ICH = intracerebral haemorrhage, SAH = subarachnoid haemorrhage, ICLAD = intracranial large artery disease.

Table 2.1: Summary of studies included in the literature review of aortic stiffness and stroke.

2.4 Carotid Artery Stiffness and Stroke

Three articles were identified on the association between carotid artery stiffness and stroke (one cross-sectional (123) and two case-controlled (124,125) studies). All authors have reported their objective(s) clearly.

2.4.1 Outcome Measures

In the cross-sectional study, Dijk et al (123) investigated the association between carotid artery stiffness and stroke in well-functioning individuals. In the case-controlled studies, Dijk et al (124) and Tsivgoulis et al (125) investigated the association between carotid artery stiffness in patients with ischaemic stroke and its risks respectively.

2.4.2 Characteristics of Study Population

Dijk et al (123,124) recruited patients who were referred to the hospital with cardiovascular disease, as part of a larger single centre cohort study for manifestations of arterial disease. Similarly, Tsivgoulis et al (125) recruited patients who were admitted to the acute stroke unit. All studies recruited patients with mean age between 60 to 65.

2.4.3 Assessment of Carotid Artery Stiffness

All studies measured carotid artery distensibility as a surrogate marker of arterial stiffness. Dijk et al (123,124) measured distensibility using ultrasonography with a wall-track system whereas Tsivgoulis et al (125) used high-resolution B-mode ultrasonography. Distensibility was calculated as the change in diameter of the common carotid artery during a complete cardiac cycle. A lower distensibility represents a stiffer artery.

2.4.4 Association between Carotid Artery Stiffness and Stroke

In a cross-sectional study, Dijk et al (123) demonstrated that a stiffer carotid artery is associated with stroke following adjustment for confounders. However, the results were expressed in regression coefficient and 95% CI without the precision of the result (-29.7, 95% CI -43.5 to -15.8).

In the case-controlled studies, Tsivgoulis et al (125) showed that carotid artery distensibility is independently associated with ischaemic stroke (OR 1.59, 95% CI 1.22-2.07, P=<0.05). In addition, Dijk et al (124) showed that patients in the lowest quartile of carotid artery distensibility had 2.1 times higher prevalence of previous TIA or ischaemic stroke compared to highest quartile of carotid artery distensibility following adjustment for blood pressure (OR 2.1, 95% CI 1.1-4.1).

First author, year	Design	Mean age	Assessment of arterial stiffness	Characteristic of patients	Outcome measure(s)
Dijik, 2004	CC	63.3±9.1	CCA dist	IS/TIA	IS and TIA
Dijik, 2004	CS	60.7±10.3	CCA dist	Stroke	Acute IS
Tsivgoulis, 2005	CC	64.8±10	CCA dist	IS	All strokes

CC = Case-controlled, CS = Cross-sectional, CCA dist = Common carotid artery distensibility, IS = ischaemic stroke, TIA = Transient ischaemic attack.

Table 2.2: Summary of studies included in the literature review of carotid artery stiffness and stroke.

2.5 Summary

A review of the literature has revealed that there are some evidence on the association between aortic stiffness and stroke. Nonetheless, only a few studies (80,111,113,115,119) have demonstrated a strong association. A major limitation of these studies was the nature of cross-sectional design. In addition, the association between aortic stiffness and stroke could be underestimated by the selection of young subjects (10,111,113,116), a significant number of exclusion due to incomplete baseline data (115), small sample size (111,118,119), highly selective study participants (10,80,112,114) and unreported estimated effect size and precision (36,114,118,120).

The scarcity of evidence on carotid artery stiffness and stroke may be due to that assessment of local arterial stiffness requires a high degree of technical expertise and longer acquisition time (7). Furthermore, measurement of local arterial stiffness is mostly performed for mechanistic analyses in pathophysiology, pharmacology and therapeutics, rather than for epidemiological studies (7).

Three studies have recently (126-128) demonstrated the feasibility of measuring carotid PWV with MRI. However, these were conference proceeding and therefore unpublished. The results of these studies shall be discussed further in the Chapter 4. MRI allows imaging plane to be placed perpendicular to the artery and data may be acquired simultaneously in two planes. Moreover, it enables precise measurement of the path length. The transit time may be difficult to determine as the distance between the two

traveling waves is short.

The process of atherosclerosis is not homogeneous, hence the result of aortic PWV should not be assumed to be applicable to local arterial stiffness. Nagai et al (129) demonstrated that common carotid artery stiffness is only moderately associated with aortic stiffness (R^2=0.42, p<0.001). The proportion of elastin to collagen is different between the aorta and the carotid arteries, which might lead to different degree of stiffness. Paini et al (130) concluded that the strength of the correlation between aortic and carotid stiffness becomes weaker as the number of cardiovascular risk factor increases (in normotensive, R^2=0.41; in hypertensive, R^2=0.16; and in both hypertensive and diabetic, R^2=0.11). Therefore, aortic and carotid stiffness should not be used interchangeably in high risk patients (111,130). Tomonori et al (131) and Nakano et al (120) have found that the increase in aortic PWV was associated with the existence of carotid plaque but not its severity. Shen et al (132) observed a similar result where cfPWV was significantly correlated with carotid intimal medial thickness but not the severity of carotid stenosis. These studies have strongly supported the need to study local arterial stiffness and arterial stiffness may be independent of the severity of intra-luminal stenosis.

In the following chapter, we shall describe the methods we have used to develop carotid PWV using MRI. We also describe the methods of MRI detected IPH as it is an important part of this thesis.

Chapter 3

Methods

3.1 Introduction

This chapter describes the common process used in patient recruitment for all cohort of subjects reported in this thesis, the MRI protocol for PWV and IPH imaging. The details on the development and analysis of PWV and IPH are also described in each section respectively.

3.2 Recruitment

Patients referred from primary and secondary care units to the one stop vascular clinic at the Queen's Medical Centre, Nottingham for assessment of their carotid arteries were identified. The routine clinical care for all patients include a thorough clinical assessment by a neurologist, stroke physician or vascular surgeon to determine the nature of the clinical presentation. Patients were approached for participation in the study if they have carotid stenosis of 50% or more on Duplex ultrasound, regardless of whether symptomatic or asymptomatic. For patients with symptomatic carotid stenosis scheduled for CEA in the next operating list, every effort was made to arrange MRI scan prior to surgery without delaying clinical care. All patients were commenced on best medical therapy following their initial assessment.

Prior to the recruitment of all prospective subjects by the author, approval was obtained from the local research ethics committee as well as research and development committee to conduct the research project. All subjects have given written consent for their participation and were given verbal as well as written information on the details of all aspects of the project as approved by the ethics committee.

3.2.1 Clinical Participant Recruitment

The inclusion criteria for patients in this study include:

- Carotid stenosis of 50% or more at the bifurcation using established Duplex scanning criteria (133).
- Patients who were either symptomatic or asymptomatic. Patients who had symptomatic ipsilateral carotid stenosis were those presented with anterior circulation events, including TIA, amaurosis fugax and stroke.
- Able to attend the University of Nottingham to undergo MRI scanning.

The exclusion criteria for patients in this study include:

- Claustrophobia.
- Any contraindication for MRI.
- If MRI might delay clinical care.

3.2.2 Healthy Volunteer Recruitment

The inclusion criteria for health volunteers include:

- Age of 60 years or above.
- No evidence of carotid stenosis at the bifurcation on Duplex scan.
- Presence of hypertension.
- No previous history of stroke, TIA, angina or myocardial infarction.
- Able to attend the University of Nottingham to undergo MRI scanning.

The exclusion criteria for healthy volunteers in this study include:

- Claustrophobic.
- Any contraindication for MRI.
- Previous carotid endarterectomy.

All informed and written consent were obtained by the author.

3.2.3 Clinical Assessment

All participants recruited were clinically assessed by the author and one of the following specialists: neurologist, stroke physician or consultant vascular surgeon. For participants with ipsilateral symptomatic carotid stenosis, medical therapy was given as clinically indicated. In general, patients were commenced on an anti-platelet agent, a statin and an anti-hypertensive if indicated.

The baseline characteristics of all study participants were recorded using a standardised pro forma to identify any cardiovascular risk factors for stroke. All participants were required to fill in a MRI safety questionnaire to ensure that they did not have any contraindications to undergo MRI scanning. Following that, MRI was performed on the carotid arteries to assess arterial stiffness and presence or absence of IPH. The details of each study are given in the respective chapters.

The following definitions were used in the clinical assessment and recording of baseline characteristics of all study participants:

Stroke was defined as rapidly developing clinical signs of focal (at times global) disturbance of cerebral function, lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin.

Transient ischaemic attack was defined as stroke symptoms and signs that resolved within 24 hours.

Hypertension was defined as the presence of systolic blood pressure of 140 mmHg or more, or a diastolic blood pressure of 90 mmHg or more on repeated measurements by the general practitioner, or when there is a history of medical treatment for hypertension.

Atrial fibrillation is defined by the presence of atrial fibrillation or paroxysmal atrial fibrillation on electrocardiography (ECG).

Chronic obstructive pulmonary disease was considered present if there was a previously documented diagnosis.

Diabetes mellitus was considered present if there was a previously documented diagnosis, current use of insulin or oral hypoglycaemic agent.

Ischaemic heart disease was defined by the presence of history of angina or myocardial infarction.

Hypercholesterolaemia was defined by the presence of total cholesterol level of 5.5 mmol/l or more, or if the patients were on statin for the treatment of hypercholesterolaemia.

Peripheral vascular disease was considered to be present if the patient had lower limb claudication or a history of peripheral vascular procedures.

Smoking was considered present if the patient is currently smoking, or absent if never smoked. The patient is considered an ex-smoker if he/she had stopped smoking for more than six months.

3.3 Magnetic Resonance Imaging

Following clinical assessment and the MRI safety questionnaire, all participants underwent MRI of the carotid arteries.

MRI scans were performed with the Discovery MR750 3.0 Tesla (General Electric, Connecticut, USA) at the University of Nottingham.

All study participants were positioned supine in the scanner. They were provided with earplugs and an emergency buzzer in case the participant wishes to terminate the scan at any point. Serial blood pressure was measured using MRI compatible digital sphygmomanometer every five minutes on the right brachial artery whilst the participants were in supine position in the MRI scanner. Four-lead electrocardiography was placed on the chest for continuous monitoring of heart rate and cardiac gating for cine phase contrast (will be discussed below). A 16-channel head, neck and spine coil was used for MRI scan in all study participants.

All MRI scans were performed by an experienced MRI radiographer (AF) trained in operating the Discovery MR750 3.0 Tesla.

Following the acquisition of images, all data was written to an optical disc (CD or DVD) to be transferred and stored in the workstation and secured server for post-processing and analysis. All study participants were coded and any identifiable details were anonymised.

3.4 Development of Magnetic Resonance Imaging of Pulse Wave Velocity

3.4.1 Introduction

MRI has been validated (121) for assessing PWV in the aorta by utilising velocity encoding imaging technique (45,107,134-143). Nonetheless, its application in medium size arteries has never been previously published. Despite the lack of published evidence in the literature, Hardy et al, (126) Kroner et al (128) and Keenan et al (127) have demonstrated the feasibility of acquiring PWV over a very short segment in the carotid arteries in healthy volunteers. The details on the development and the current evidence on the assessment of PWV have been discussed in Chapter 1.

Velocity offset has been a known issue in cardiovascular flow quantification using MRI. The cardiovascular magnetic resonance (CMR) working group of the European Society of Cardiology has recently investigated the implication of velocity offset in the quantification of cardiopulmonary flow (144). The study showed that none of the CMR systems were consistent below the proposed maximum acceptable offset value (0.6 cm/s) (144). Several authors (145-148) have since attempted to address this problem by either sequence optimisation or acquiring static phantom images. Nevertheless, in order to improve the accuracy, a baseline measurement of a static object that has zero flow is desirable. This mitigates phase errors which may be introduced by eddy

currents and can add to or subtract from the flow measurement.

This study does not measure the absolute value of velocity and therefore the effect of velocity offset in determining transit time of pulse waves may be negligible. However, the results of the studies above undoubtedly raised uncertainty on the precision of MRI PWV. Hence, we have undertaken static phantom scanning to correct baseline measurement and also to investigate the significance of velocity offset in MRI PWV. We have also measured aortic arch MRI PWV to determine if it was association with carotid artery stiffness as reported in the literature (149).

3.4.2 Magnetic Resonance Imaging of Pulse Wave Velocity

I. Protocol

Carotid PWV was acquired using cine phase contrast (Cine-PC) (known as FastCine sequence in the GE Discovery MR750). It is a scan mode that uses a pulse sequence diagram from the gradient echo family with cardiac gating imaging option and a value entered for the number of phases to reconstruct the cardiac tab. The pulse sequence diagram can be fast gradient echo or spoiled gradient echo of fiesta acquisition with the number of cardiac phases to reconstruct set to a specific value, typically 20 to 24. It is acquired in the 2D mode. FastCine allows segmented k-space which allows for short breath-hold scan times. The k-space segmenting technique reconstructs all phase steps regardless of when they are acquired within the cardiac cycle. This allows for complete imaging of the R-R interval allowing better visualisation of end diastolic events. The variable view sharing technique reconstructs images at different delay times within each cardiac cycle depending on a temporal phase position that varies with the heart rate. It combines the strengths of fast card and cine for shorter scan times and imaging through the cardiac cycle.

Following localisation of the carotid arteries, a three dimensional time of flight (ToF) was acquired to identify the carotid bifurcations and the course of the carotid arteries. Cine-PC was used to measure the flow in the carotid arteries. Prior to the acquisition of Cine-PC, all study participants have rested in supine position for at least 15 minutes with the MRI room temperature set between

20 to 21 degree Celsius.

Cine-PC was acquired axially using one-directional through-plane phase velocity mapping by applying a single plane transecting the common carotid and the internal carotid arteries to determine the transit time between them. Each plane was acquired separately to ensure it was perpendicular to the arterial wall to maximise the encoding of through-plane velocity. The plane transecting the internal carotid arteries was position approximately 24mm (three slices) above the carotid bifurcation on the ToF image and then angled perpendicular to the arterial wall. The plane was viewed on at least two ToF images and crossed referenced with the localising images in sagittal view to ensure the plane was perpendicular to the wall of the carotid arteries. Similarly, the slice transecting the common carotid arteries was positioned approximately 24mm below the carotid bifurcation and the same steps were taken to ensure it was perpendicular to the arterial wall. Data acquired at each plane was interleaved for 80 cine frames within each cardiac cycle and repeated with velocity encoding on the subsequent cycle. The method used to measure carotid PWV was also used to measure aortic arch PWV. The proximal end of aortic arch was defined anatomically at the level of sternomanubrium junction and the distal end at the level of T4 vertebra. Aortic arch PWV was measured to validate carotid PWV.

Static phantom was scanned at the end of protocol in healthy volunteers to assess the effect of velocity offset in carotid PWV. Healthy volunteers were removed from the MRI scanner without undocking the table to preserve the landmark. Round static phantom was placed in the coil and heart rate was

emulated at 100 beats per minute. The Cine-PC was acquired at the same plane as before.

The following sequence was used to acquire Cine-PC axially at each plane:

TR 5.88 ms, TE 3.032 ms, FA 15 degrees, acquisition matrix 192x192 with 80% FOV, velocity encoding range (VENC) 85 cm/s, acquisition time 60 to 90 seconds depending on the heart rate.

II. Post-Processing

Following acquisition of the MRI images, the Cine-PC images were saved in a compact disc and subsequently transferred to a workstation for analysis.

Image analysis for MRI Cine-PC was performed on Advantage Workstation AW 4.3_05 (General Electric, Connecticut, USA). CV Flow V3.3 by Medis was used to analyse the flow and velocity data acquired through-plane in each carotid artery.

The author had undergone training of analysing Cine-PC using CV Flow by the senior process development technician (SR) from General Electric Healthcare.

III. Analysis of MRI PWV

A. Determining the distance travelled by the pulse waves:

The length of the carotid arteries (distance) travelled by the pulse waves was measured on the workstation. Maximal intensity projection ToF images were used to measure the distance by cross-referencing with the Cine-PC images to determine the level of image acquisition. The distance between the two levels was measured along the middle path of the artery. At least two measurements were taken at two different views on the maximal intensity projection ToF images. The mean of the two measurements (in meters) were used as the distance travelled by the pulse waves.

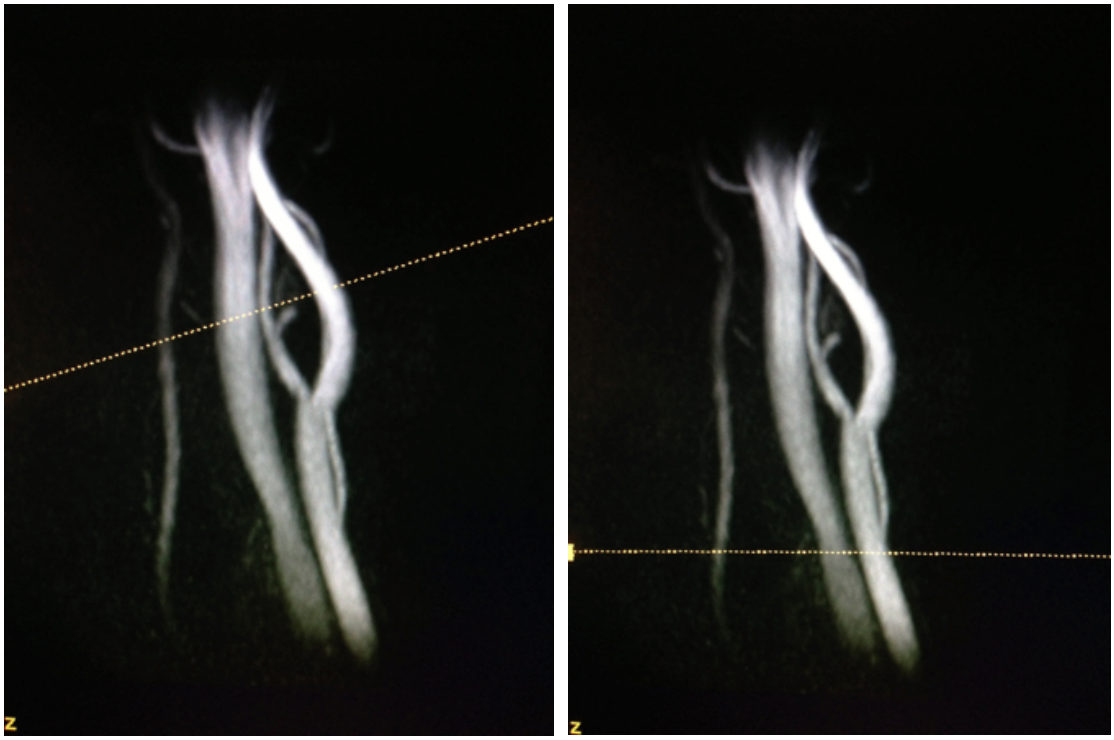


Figure 3.1: The maximal intensity projection ToF images were crossed referenced with the cine-PC series to identify the level of the carotid artery where Cine-PC was acquired

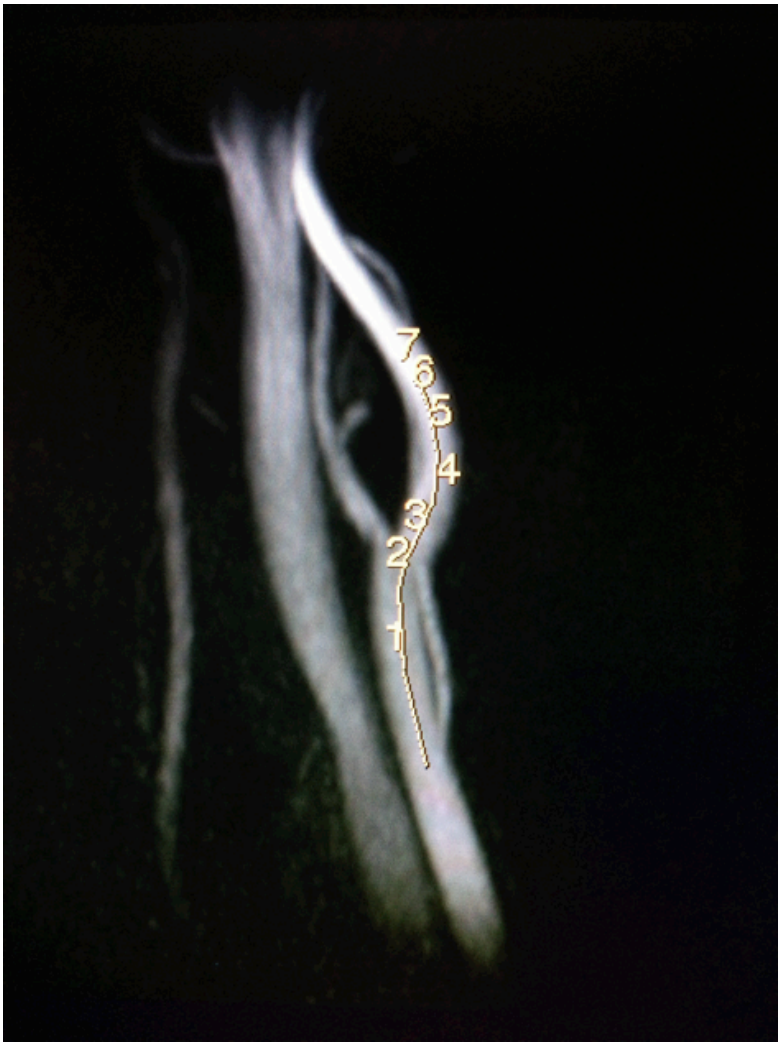


Figure 3.2: Following identification of the Cine-PC planes, the distance between the two planes was measured along the middle path of the artery.

B. Determining the transit time between the pulse waves:

The transit time between the pulse waves was determined by plotting the velocity-time curves from the data acquired with Cine-PC at the CCA and ICA. The Cine-PC images were analysed offline using CV Flow 3.3 by Medis (Leiden, Netherlands) in the Advantage Workstation AW 4.3_05 (General Electric, Connecticut, USA). Upon the loading of the axial Cine-PC images (80 images) on to CV Flow, the CCA or ICA was identified by cross referencing with the axial view of ToF and magnified between 300% to 500% on the image taken at peak systole. This was to ensure the intra-luminal area was correctly identified and to minimise the inclusion of arterial wall in to the region of interest (ROI). ROI was then drawn around the CCA or ICA on the modulus image of peak systole. This was done by selecting the isocentre of the CCA or ICA and the ROI was drawn around the intra-luminal area using automatic contour detection. This was based on motion detection with the maximum motion set at 10 pixels. The ROI was then propagated to the rest of the images automatically. The modulus and phase images were played in a movie to ensure that the ROI stays in the same position throughout the images. In cases where the ROI was deviated from intra-luminal area, it was deleted and redrawn using the same method. A preview of the velocity-time curve was generated and the report was saved in a tab delimited text file, which was then transferred and converted to Microsoft Excel file.

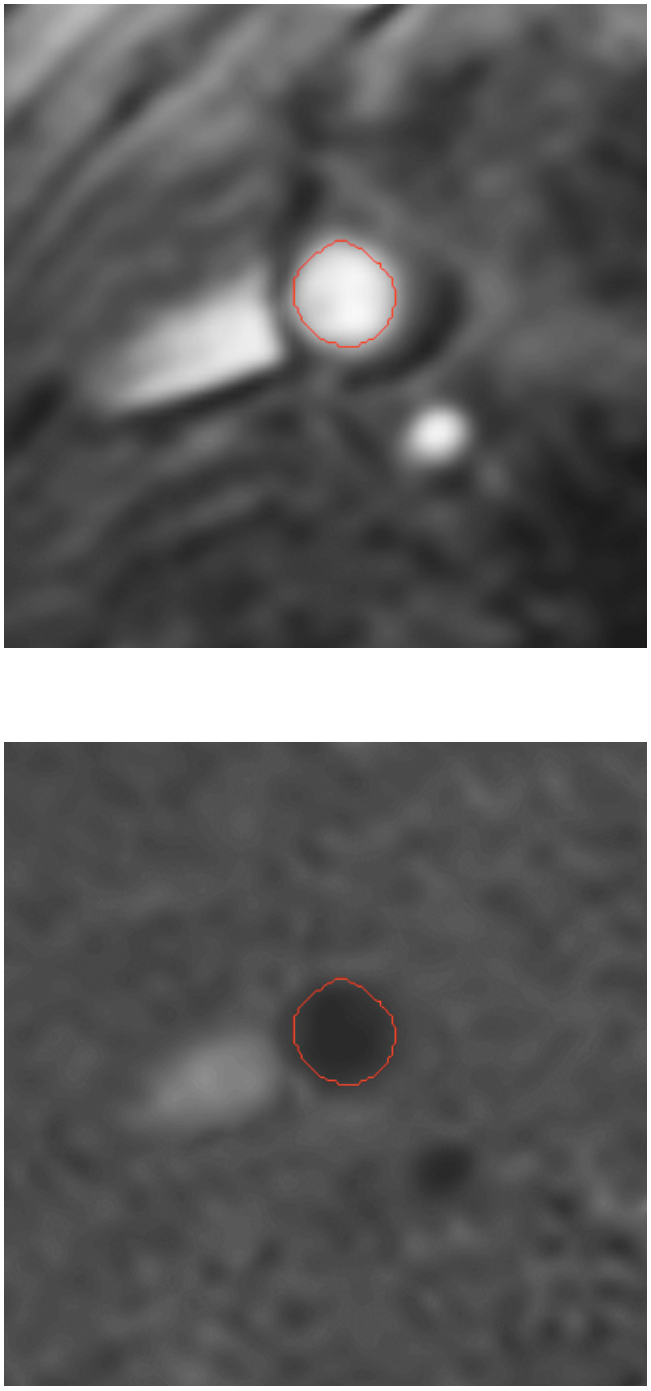


Figure 3.3: An example of drawing ROI. A right CCA was magnified at 500% on CV Flow. The ROI was drawn on the modulus (top) by selecting the isocentre of the intra-luminal area. This was followed by automatic motion detection (red line) to complete the ROI.

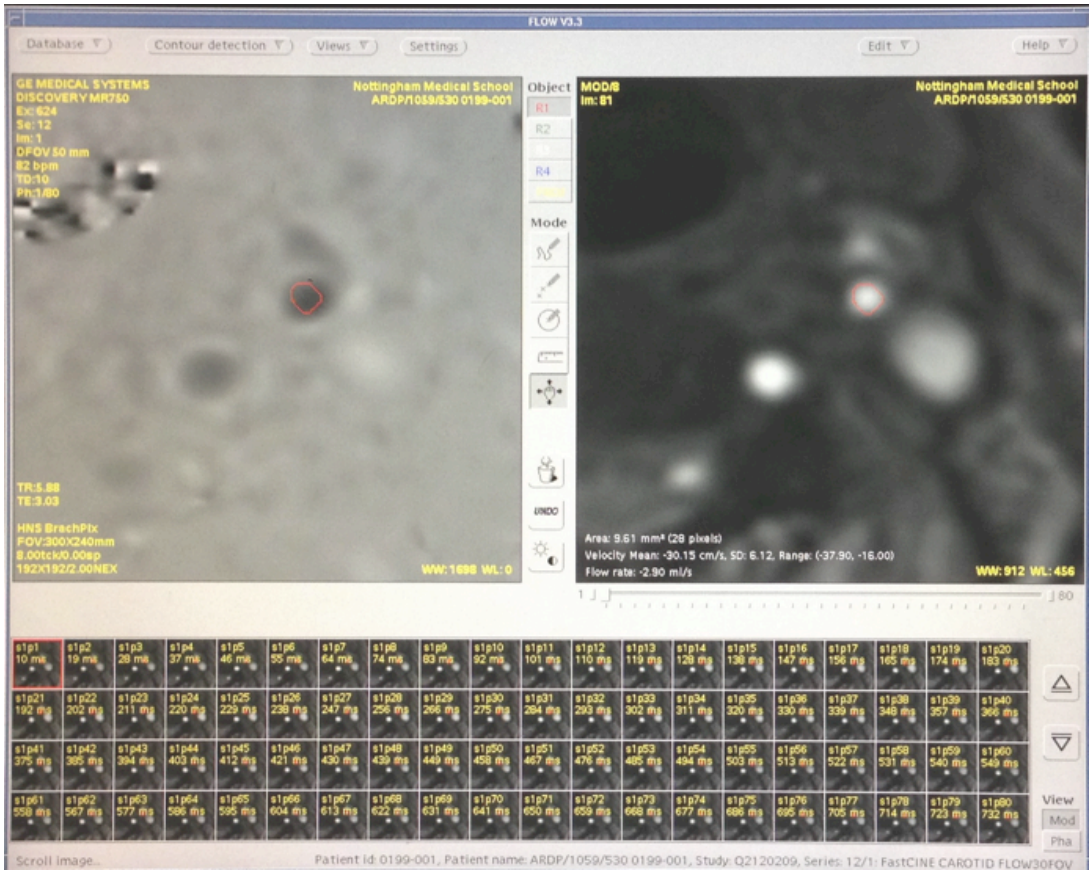


Figure 3.4: ROI was drawn on the modulus image (top right), which was replicated on the phase image (top left). The ROI was subsequently propagated automatically to all images (bottom) and a movie was played to ensure the ROI stays in place.

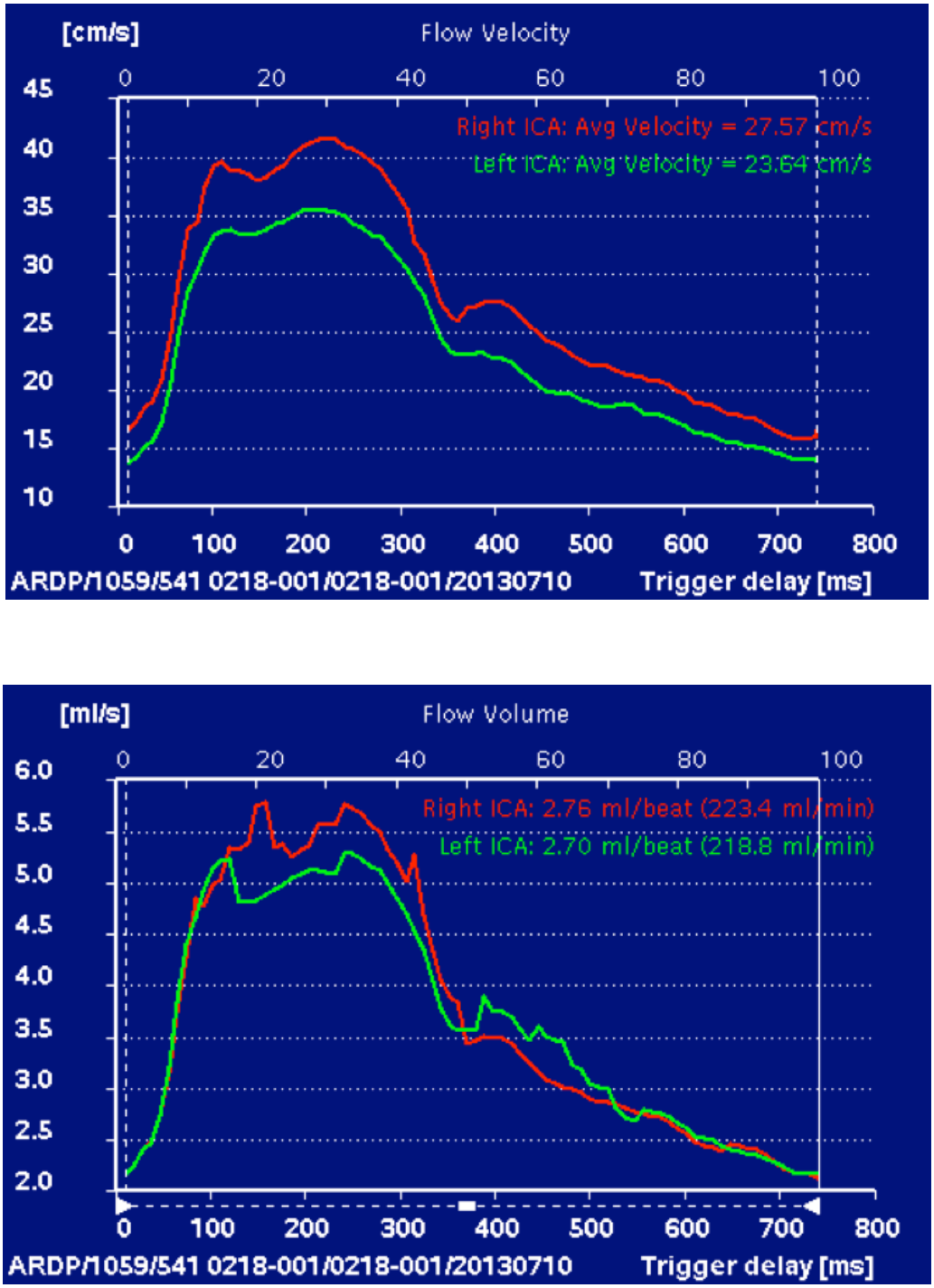


Figure 3.5: A preview of velocity-time curve and flow-time curve generated in CV Flow based on the ROI drawn in both ICA.

Phase Nr	Trigger [ms]	Area [mm2]	Area [#pix]	Vel [cm/s]	SD Vel [cm/s]	Min V [cm/s]	Max V [cm/s]	Flow [ml/s]
1	10	35.01	102	12.19	4.06	18.9	1.8	4.34
2	25	35.01	102	16.91	4.85	24.5	6.5	6.08
3	41	36.04	105	28.57	7.87	40.5	6.1	10.62
4	56	38.1	111	31.96	10.28	47.5	7.7	12.69
5	72	36.73	107	35.2	10.64	51.9	10.8	13.64
6	88	40.51	118	32.6	13.49	54.1	5.9	14.13
7	103	40.16	117	31.5	13.61	53.2	5.3	13.77
8	119	41.88	122	28.62	14.34	51	0.5	13.29
9	135	40.85	119	28.53	13.87	50.2	0.1	13.14
10	150	41.88	122	27.75	13.7	49.3	0	13.29
76	1181	30.89	90	7.3	3.04	13.2	2.2	9.11
77	1197	32.27	94	6.61	3.1	12.6	1.6	9.02
78	1213	31.58	92	6.52	2.96	12.2	1.5	8.98
79	1228	32.27	94	6.77	2.96	12.4	1.6	9.15
80	1244	33.98	99	9.63	3.33	15.7	2.7	10.28

Figure 3.6: An example of raw data obtained from a Cine-PC.

The data in Microsoft Excel format were transferred to a desktop connected to the network at the University of Nottingham. Mean velocity (centimetre per second) was used to plot against the trigger time (millisecond) to generate the velocity-time curve acquired at both CCA and ICA. The transit time between the two waves (pulse wave at the CCA and ICA) was determined in order to calculate the carotid PWV. There are several methods to determine the transit time. Even though there are no general consensus as to which is the most methodologically robust method, the foot-to-foot method was commonly used and therefore our first choice to determine the transit time. (150,151) In addition, we have used the sigmoid model to determine the transit time in case the first method fails, as reported by some authors (152,153). The foot of the curve represents the beginning of systole in a cardiac cycle and thus the beginning of the pulse wave. This was done by determining the point yielded by the intersection between the horizontal line passing through the minimum

point and the linear regression modelled from the systolic up slope of the velocity wave. The regression line was modelled from the velocity values between 10% and 30% of the total range. For the sigmoid model, the systolic up slope of the normalised velocity waveforms on the CCA and ICA were fitted to a sigmoid curve respectively using the least squares minimisation approach. The transit time was calculated using the equation below (150).

$$\Delta t = \Delta t_k \,/\, Er(\Delta t_k) = 0 .$$

Where:

$$Er(\Delta t_k) = \int_{t_1}^{t_2} \left(Seg_A(t) - Seg_D(t - \Delta t_k) \right) dt .\ \Delta t_k \in \Re$$

An in-house written script in Matlab 7.12.0.635 (The Mathworks, Natick, MA, USA) was used to calculate the transit time using the foot-to-foot and the sigmoid model method. The results were converted from milliseconds to seconds.

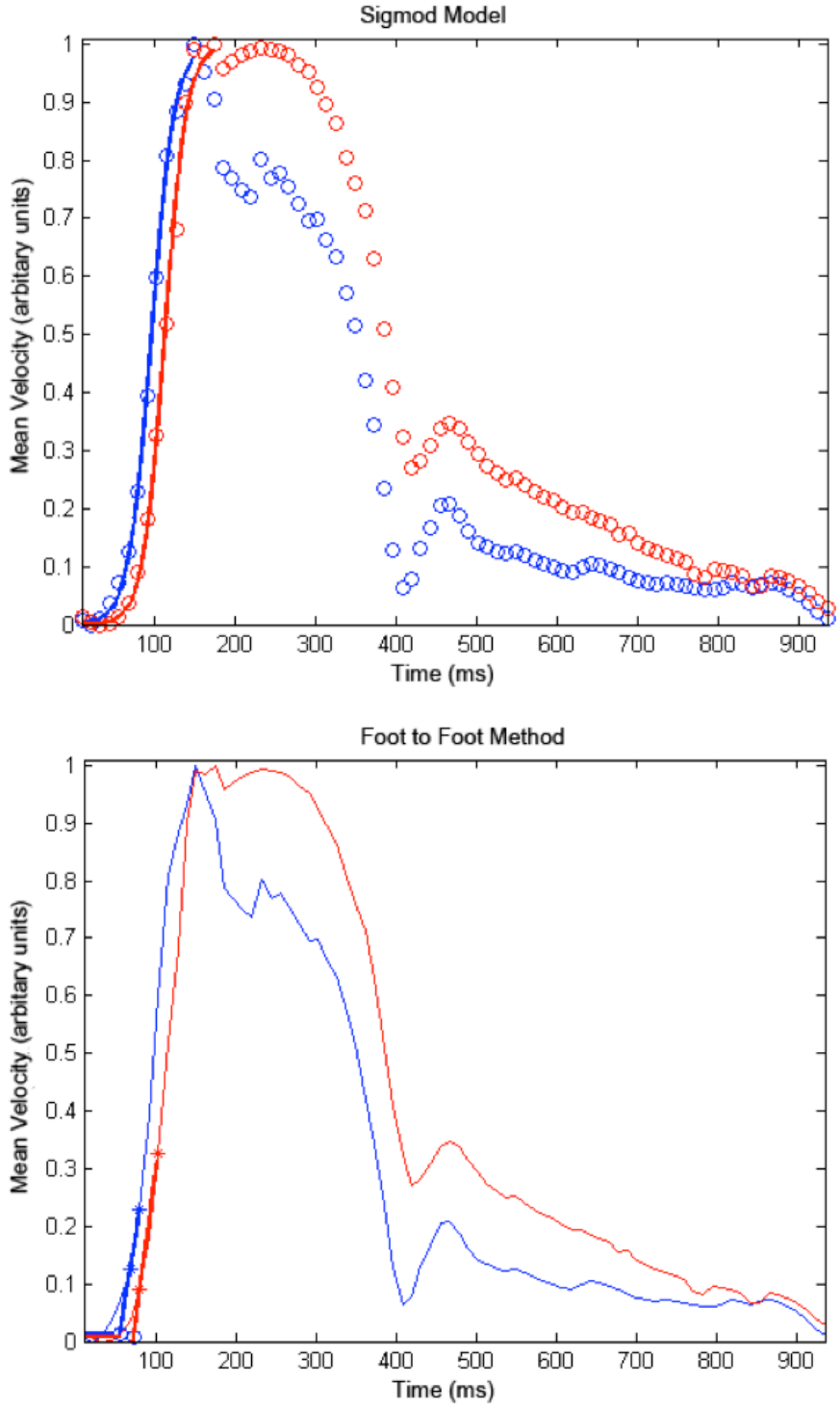


Figure 3.7: Two methods used to determine the transit time of the pulse waves. Blue line represented CCA whereas red line for ICA.

Once the distance and the transit time were obtained, the PWV of the carotid arteries was calculated with the following formula.

$$PWV(m/s) = \frac{d(m)}{t(s)}$$

PWV was expressed in meters per second. It was calculated by dividing the distance (d, in meters) of the carotid artery travelled by the pulse wave with the transit time (t, in seconds) between the arriving pulse wave at CCA and ICA.

C. Phantom correction for background velocity offset:

Baseline measurement to correct for velocity offset was performed by copying the ROI from the carotid arteries and placed on to the phantom images. This was then analysed using the same method as described before. The velocities obtained from the phantom images were subtracted from the velocities obtain from Cine-PC to correct for any offsets.

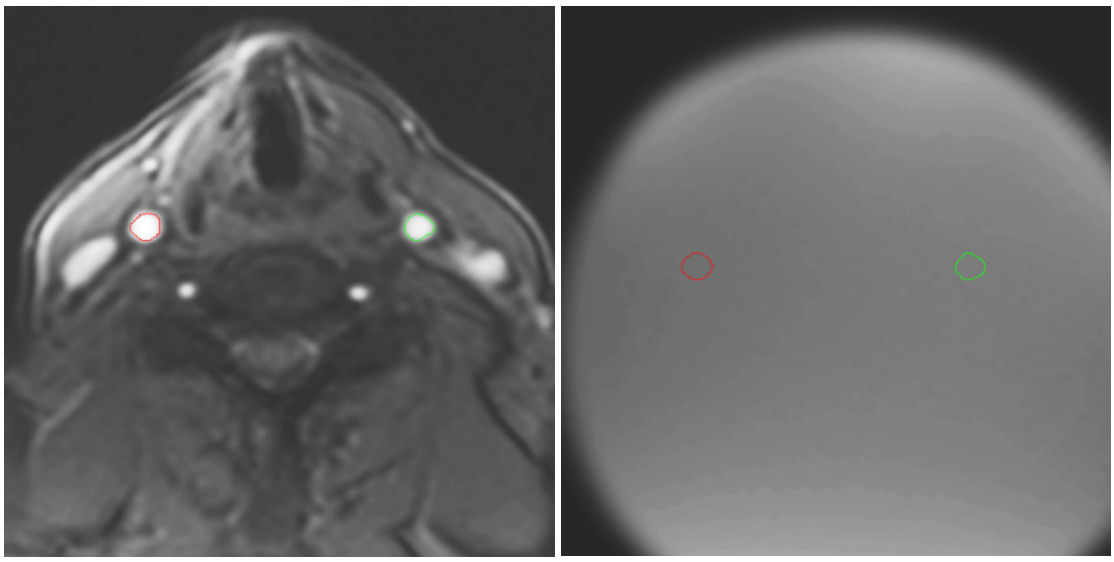


Figure 3.8: An example of correcting velocity offset. ROI drawn on the CCA (left) were copied and placed on to the phantom image (right) to obtain the velocity offset from the phantom image at the same location. The values of offsets were manually subtracted to correct any offsets.

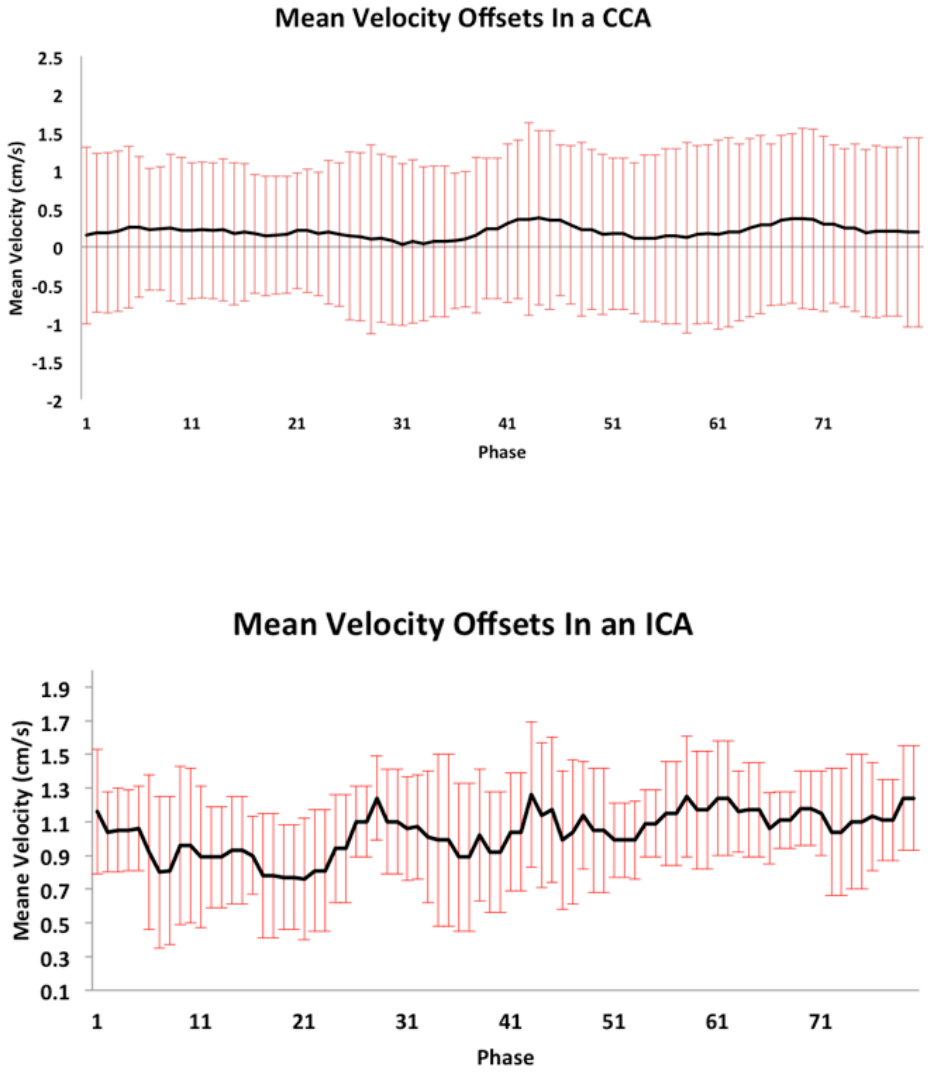


Figure 3.9: An example of velocity offsets in a CCA and ICA. Vertical red lines represent standard deviations.

3.4.3 Quality Assessment

MRI PWV is a novel technique to quantify arterial stiffness, particularly in the carotid arteries. We have therefore performed analyses to assess the reliability and consistency of this new imaging technique. Both the imaging protocol and methods of analysis have been described previously in Chapter 3.4.2. In addition, we have performed PWV of the aortic arch to validate the association reported in the literature between the carotid and aortic stiffness. All statistical analyses were performed using IBM SPSS Statistics (Version 21; Chicago, Illinois).

For the assessment of intra-scan repeatability (test-retest reliability), Cine-PC was acquired twice at each plane. During analysis with CV Flow, the ROI drawn on the first Cine-PC series was used as the ROI in the repeated Cine-PC series. In cases where the ROI had deviated in the repeated series (due to movement during acquisition), a new ROI was drawn. For the assessment of intra-rater consistency, each selected Cine-PC series was analysed twice by re-drawing the ROI at the same region. Furthermore, PWV results after correcting background noise with static phantoms were compared with PWV results without phantom correction. This was to assess the effect of background noise in the assessment of carotid PWV.

Both intra-scan repeatability and intra-rater consistency were analysed with both intra-class correlation and Bland-Altman plot. Wilcoxon signed-rank test was used to compare carotid PWV values with and without static phantom

correction.

Finally, we examine the association between aortic arch PWV and carotid PWV. Evidence in the literature have shown that there is an association between carotid and aortic stiffness. We aim to examine if this association is present in this study. We then attempt to use aortic arch PWV to validate carotid PWV by examining the association between carotid and aortic arch PWV in patients with carotid artery stenosis using multiple linear regression. The median of both PWV were then compared using Mann-Whitney U test to determine if there was any difference.

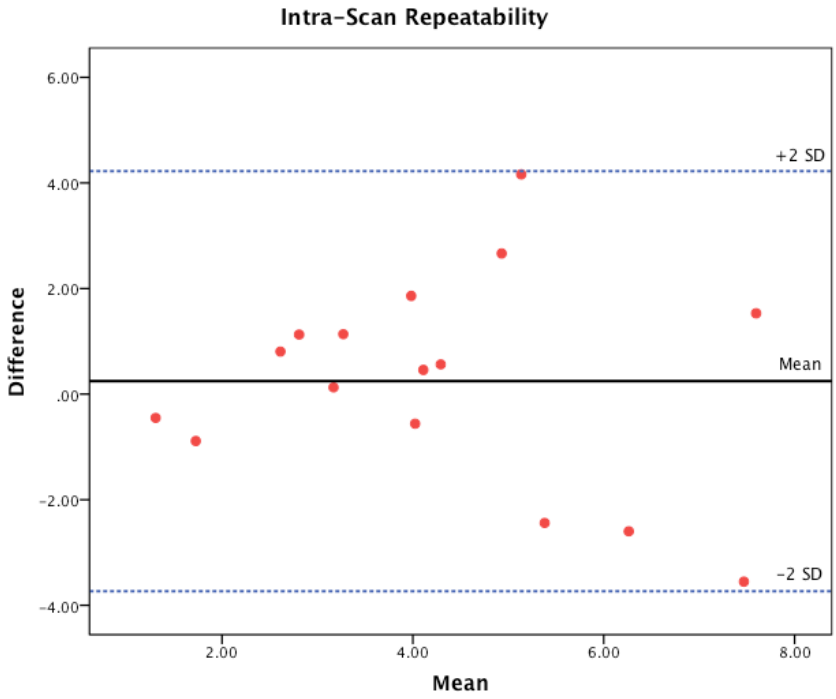
I. Intra-scan repeatability

Eight study participants were randomly selected to undergo repeated Cine-PC acquisition to assess intra-scan repeatability. A total of 16 carotid arteries were included in the analysis. The ROI drawn on the first Cine-PC image of each study participant was saved and loaded on to the repeated Cine-PC where possible. In cases where the participant had moved during image acquisition resulting in mismatching of the ROI between the first and the repeated Cine-PC image, a new ROI was drawn on the repeated image. After plotting the velocity-time curve, the foot of the curve was determined by the first rise in velocity after diastole. Transit time between the two curves was determined using the methods described in Chapter 3.4.2 (III), The results of PWV are shown in the table below.

First scan		Second scan	
Time (ms)	PWV (m/s)	Time (ms)	PWV (m/s)
12.272	4.34	13.721	3.88
13.216	4.57	15.072	4.01
14.366	4.96	9.429	7.56
9.460	8.36	11.579	6.83
16.553	3.23	17.228	3.11
11.463	3.84	16.279	2.70
16.848	3.02	23.000	2.21
14.600	3.74	12.700	4.30
8.400	6.26	14.619	3.60
15.300	3.37	22.991	2.24
14.263	4.16	8.991	6.60
12.166	5.69	7.491	9.24
38.422	1.28	22.702	2.17
42.590	1.08	30.070	1.53
8.824	4.91	14.200	3.05
7.808	7.22	18.450	3.05

Table 3.1: The results of PWV in 16 carotid arteries for the assessment of intra-scan repeatability.

Cronbach's Alpha for intra-scan consistency was 0.703. This showed good agreement between two Cine-PC images acquired during the same scanning session. The result is similar to those reported by Keenan et al (127). The Bland-Altman plot is as below.



Bias	0.25
Precision (±)	1.99
Upper Limits of Agreement	+4.23
Lower Limits of Agreement	-3.74

Figure 3.10: Bland-Altman plot for intra-scan repeatability.

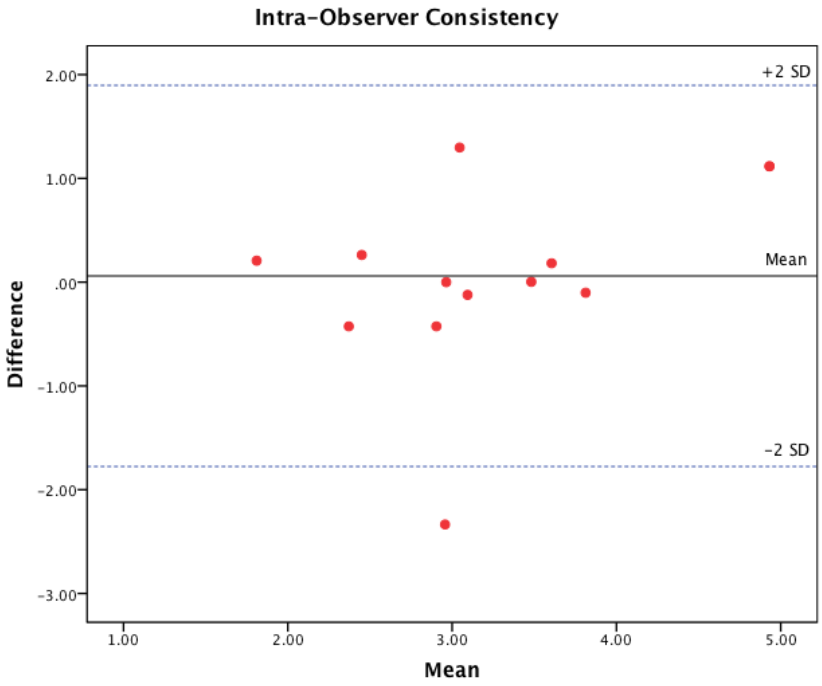
II. Intra-observer consistency

13 carotid arteries were randomly selected to undergo repeated analysis with CV Flow V3.3 for intra-rater consistency. Each carotid artery was analysed twice, with the second analysis performed approximately four to eight weeks after the first one. Following that, the velocity-time curve of each analysis was plotted to determine the transit time and to calculated PWV. The methods of determining the transit time was described in Chapter 3.4.2 (III), The results of of transit time and carotid PWV are as below.

FIrst Analysis		**Repeated Analysis**	
Time (ms)	PWV (m/s)	Time (ms)	PWV (m/s)
12.853	3.76	12.515	3.86
9.926	3.03	9.539	3.16
27.200	1.91	30.500	1.71
9.400	5.49	11.800	4.37
23.332	1.79	10.122	4.12
13.500	3.70	14.200	3.51
16.600	2.96	16.600	2.96
18.600	2.58	20.700	2.32
20.741	2.16	17.318	2.58
9.732	3.48	9.741	3.48
18.300	2.69	15.800	3.12
9.400	5.49	11.800	4.37
8.500	3.69	13.100	2.40

Table 3.2: The results of PWV in 13 carotid arteries for the assessment of intra-rater consistency.

Cronbach's Alpha for intra-observer consistency was 0.747. This showed good consistency between PWV analysis performed at two separate session. The Bland-Altman plot is shown below.



Bias	0.06
Precision (±)	0.92
Upper Limits of Agreement	+1.90
Lower Limits of Agreement	-1.78

Figure 3.11: Bland-Altman plot for intra-observer consistency.

III. Phantom correction for velocity offset

The aim of velocity offset correction with static phantom was to assess the effect of background noise on the measurement of PWV. Static phantom was scanned following the completion of protocol in seven healthy volunteers. The details of scanning static phantom has been described in Chapter 3.4.2 III (C). The results of PWV with and without correction for velocity offset are as below.

With Phantom		Without Phantom		Difference	
Time (ms)	PWV (m/s)	Time (ms)	PWV (m/s)	Time (ms)	Percentage
9.8	5.43	10.4	5.12	0.31	6
7.3	8.28	8.7	6.95	1.33	16
16.1	4.43	16.5	4.32	0.11	2
12.7	6.23	13.7	5.77	0.45	7
25.7	2.08	20.2	2.65	0.57	27
13.5	3.26	14.0	3.14	0.12	4
18.5	2.75	18.5	2.75	0.00	0
15.3	3.57	14.6	3.74	0.17	5
9.7	6.12	8.8	6.74	0.63	10
11.8	5.87	13.5	5.13	0.74	13
26.9	1.83	29.7	1.66	0.17	9
32.0	1.44	33.0	1.39	0.04	3
15.2	2.85	14.1	3.07	0.22	8
14.4	3.91	14.6	3.86	0.05	1

Table 3.3: The results of PWV in 14 carotid arteries with and without phantom correction. The difference in percentage was calculated as (the difference in transit time / transit time obtained after velocity offset correction) X 100%.

The mean difference of transit time between PWV with and without velocity offset correction was 0.12 ms. Wilcoxon signed-rank test showed the differences in carotid PWV measured with and without correction for velocity offset was not statistically significant (P=0.4).

IV. Aortic Arch Pulse Wave Velocity

16 patients have successfully underwent MRI aortic arch PWV imaging. The median aortic arch PWV was 7.1 m/s (IQR 4.4-13.2). Figure below shows an example of an aortic velocity waveform in a patient with carotid artery stenosis.

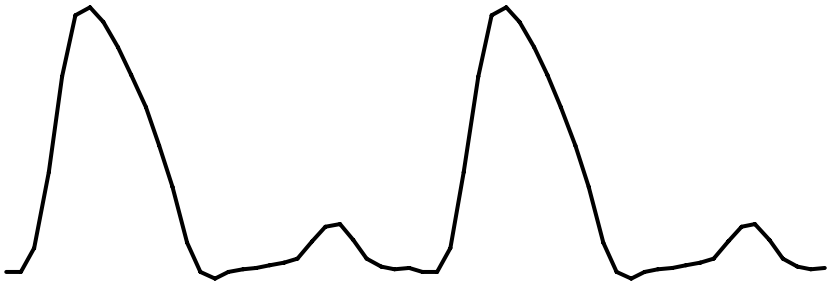


Figure 3.12: An example of aortic velocity waveform in a patient with carotid artery stenosis.

Multiple linear regression was used to examine the association between aortic arch PWV and age, blood pressure parameters as well as cardiovascular risk factors.

	R^2	P Value
Age	0.031	0.35
Male	0.018	0.47
Mean Systolic Blood Pressure (mmHg)	0.001	0.85
Mean Diastolic Blood Pressure (mmHg)	0.191	0.014
Mean Pulse Pressure (mmHg)	0.083	0.12
Mean Arterial Pressure (mmHg)	0.072	0.14
Mean Heart Rate	0.044	0.26
Chronic Obstructive Pulmonary Disease	0.005	0.7
Diabetes Mellitus	0.018	0.47
Hypertension	0.118	0.05
Hypercholesterolaemia	0.002	0.8
Ischaemic Heart Disease	0.048	0.23
Cerebrovascular Disease	0.005	0.7

Table 3.4: The association between aortic arch PWV and age, blood pressure parameters as well as cardiovascular risk factors.

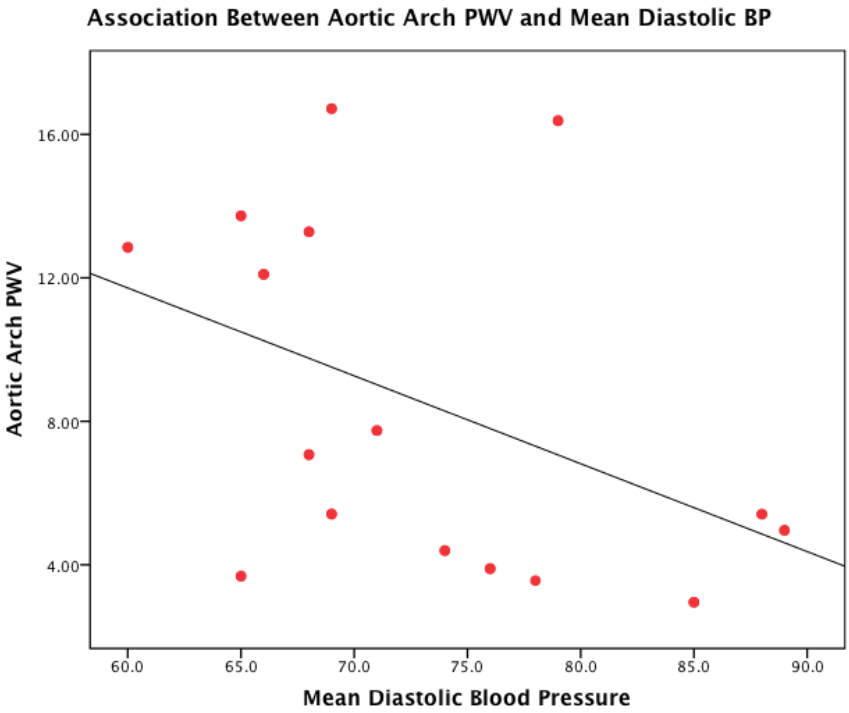


Figure 3.13: Scatter plot demonstrating the association between aortic arch PWV and mean diastolic blood pressure. R^2=0.191, beta-coefficient=-0.245, 95% CI -0.436 to -0.54, P=0.014.

V. Association Between Carotid and Aortic MRI Pulse Wave Velocity

Multiple linear regression did not demonstrate a statistically significant association between carotid PWV and aortic arch PWV (R^2 = 0.0001, P=0.9). Following adjustment for age and blood pressure parameters, the association was R^2 = 0.226, P=0.7.

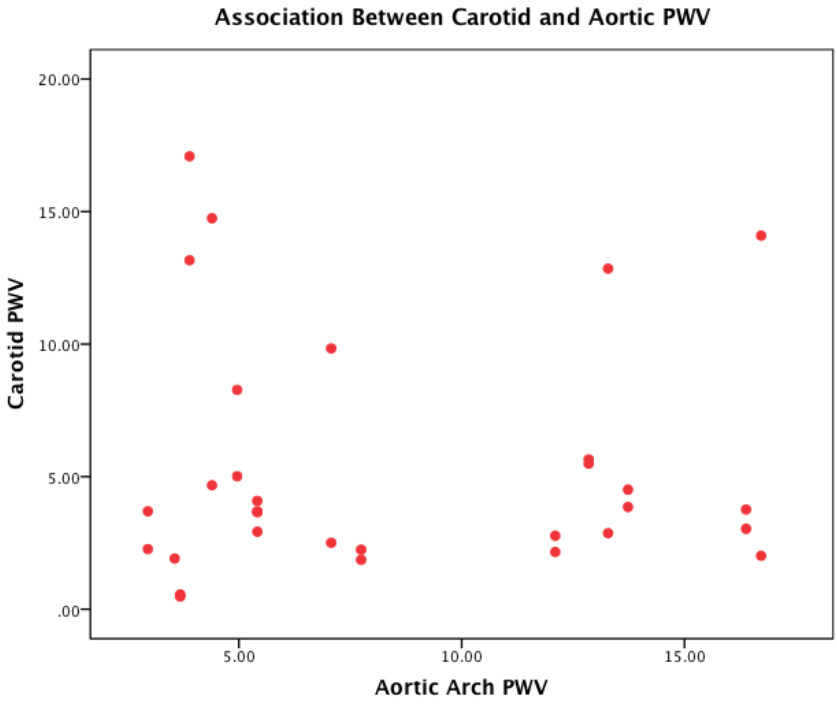


Figure 3.14: Scatter plot shows the association between carotid and aortic arch PWV in patients with carotid artery stenosis.

Mann-Whitney U test was used to compare the median of carotid and aortic arch PWV. The results showed that aortic arch PWV was statistically higher than carotid PWV. Table below summarises the results.

	Median (IQR)		
	Aortic Arch	**Carotid**	**P Value**
PWV (m/s)	7.1 (4.4-13.2)	3.7 (2.4-6.3)	0.002

Table 3.5: Comparing the median value of aortic arch and carotid PWV.

3.4.4 Discussion

Carotid PWV measured using MRI is a preliminarily feasible method of assessing local arterial stiffness. We have demonstrated that MRI acquisition of Cine-PC in the carotid arteries has a fairly good repeatability and the analysis of PWV was consistent within assessor. The acquisition time of Cine-PC was very short, approximately 60 to 90 seconds depending on the heart rate. However, carotid ToF or phase contrast needs to be performed prior to Cine-PC to determine the location of the planes. In the author's opinion, the image quality of phase contrast on the Discovery MR750 3 Tesla was poor and therefore ToF was a prerequisite. Although MRI PWV has previously been validated in the aorta (121), no studies have done so in the carotid arteries. That is perhaps one of the major limitations of this study. Validation of carotid MRI PWV could potentially be performed during cardiac catheterisation, CEA or during stenting of carotid artery. Advancing intra-arterial catheter in to the carotid arteries during cardiac catheterisation may not be ethical and doing so during CEA may increase the risk of intra-operative thromboembolism. Perhaps the most feasible method to validate carotid MRI PWV would be during stenting for carotid artery stenosis.

There were several issues regarding the MRI sequence that needs to be addressed. The electrocardiography (ECG) triggered Cine-PC acquires flow and velocity data using time averaged signal over several cardiac cycles. With the sequential acquisition of through-place velocity at both CCA and ICA, the end diastole could potentially be missed, resulting in error in determining the

exact transit time. In addition, partial volume effects and intra-voxel phase dispersion in phase contrast could lead to underestimation of peak velocity particularly in areas with turbulent flow, such as in carotid artery stenosis (154). These could be overcome by other imaging technique such as Fourier velocity encoding with the trade-off of longer acquisition time (154). Furthermore, due to the tortuosity of the carotid arteries in the elderly population, it was challenging to position the slice at absolute 90 degrees to the wall of the arteries and this may result in underestimation of the true velocity. Finally, the temporal resolution of Cine-PC may not be sufficient in assessing carotid PWV in some patients, leading to loss of data during reconstruction. Background velocity (noise) did not seem to affect the results of carotid PWV. This is likely because the measurement of PWV did not involve the absolute value of velocity but the transit time between the two traveling pulse waves. The mean discrepancy of time delay (0.12 ms) before and after correction for background velocity offsets can be considered negligible.

We have came across several challenges during the analysis of carotid MRI PWV. One of the main difficulties was to determine the rise of the pulse wave in the carotid artery. The foot of the systolic phase is often regarded as the reference point as it is thought to be free from wave reflections (13). Morever, the local velocity of blood flow that modulates the PWV independently of arterial stiffness is close to zero at the foot of the systolic phase (155). As discussed in Chapter 1, in addition to the incident wave caused by ejection of stroke volume from the left ventricle, secondary waves may contribute to the

arterial pressure waveform. These are reflected waves secondary to impedance mismatch in the arterial tree. Often these are due to the presence of bifurcation, changes in the caliber of the arteries as the pulse wave travels away from the heart and increase in arterial stiffness. Furthermore, wave reflection can be transmitted from the lower limb arterial system to the brachial and carotid arteries and appear as forward wave (156). Therefore, the accuracy and precision of the transit time measurement in human arteries is affected by reflections from within the arterial segment of interest or from other regions (157). Dissimilar to forward reflection waves, the backward propagating waves may alter the shape of the pressure waveform and subsequently its derivatives over the measured segment of the artery. This may interfere with the systolic foot and affect PWV measurement (157). Hermeling et al (157) investigated the existence of early wave reflections and their interference with systolic foot identification in CCA using ultrasonography. They found that the onset of the systolic wave is rapidly followed by a reflected wave traveling in reverse direction. Moreover, merging of the inflection point of the reflected wave and the systolic foot resulting in difficulties in the identification of the inflection point (157). It was conclude that the systolic foot identification will be affected by the interaction of the incident and reflected waves if the measurement site is high in the CCA (157).

The observations by Hermeling et al (158) indeed could be evident at times during the analysis of PWV. In aortic PWV, the foot of the wave is easy to identify at the end of diastole and is usually followed by a sharp upstroke and straight rise to the first systolic peak. In contrast, the carotid artery has a

gradual, slow rise at the end of diastole. Together with background noise obtained during MRI carotid PWV acquisition, identifying the foot of the wave can be challenging and difficult. As the result, the value of PWV can be either under or overestimated if the foot of the wave was not identified correctly. This problem was often encountered when determining the transit time using the foot-to-foot method. This can be solved by choosing another point as the foot of the wave by re-examining the velocity of the pulse wave. Alternatively, the transit time can be determined using the sigmoid method as described in Chapter 3.4.2 III B.

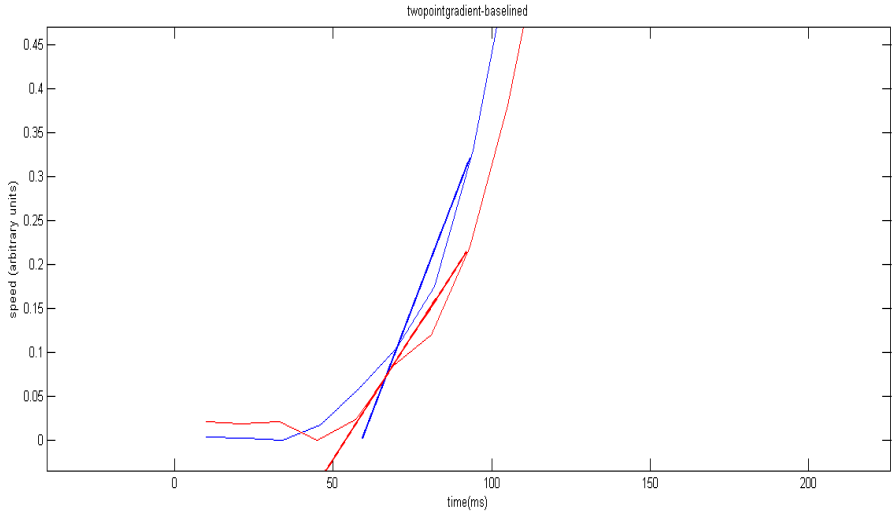


Figure 3.15: An example of error in determining the transit time. This may be attributed to slow rise of systole and MRI signal noise. Blue represents the pulse wave in the CCA whereas red in ICA. The velocity-time curves has been magnified to show the foot of the curves.

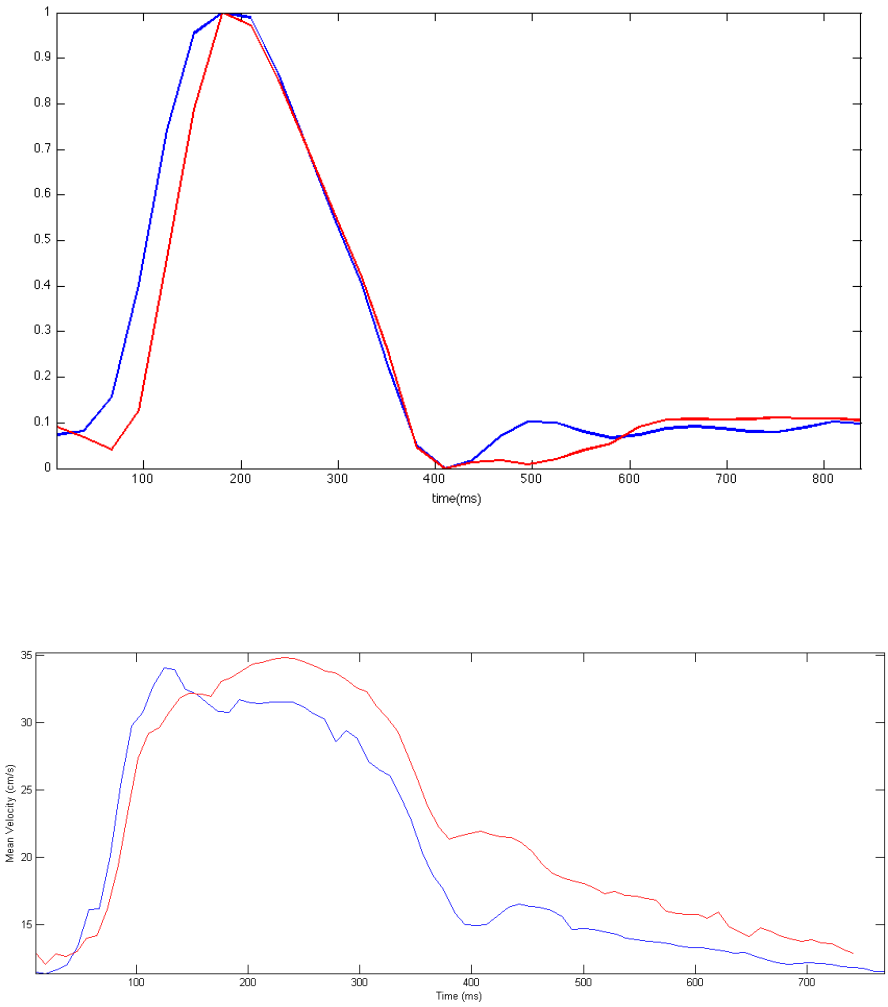


Figure 3.16: Top figure: An example of aortic waveform with sharp upstroke during systole. Bottom figure: An example of carotid waveform with gradual, slow rise at the beginning of systole. Note that the rapid systolic upstroke was distorted by reflection wave.

A further challenge encountered during the analysis was in determining the foot of the wave. As discussed, when there was difficulty or error in identifying the foot of the wave using the foot-to-foot method, an alternate point on the X axis (time) was used. This was done by choosing other point as the foot of the wave to recalculate PWV. It was found that in several cases, the value of PWV can change drastically by moving a phase forward or backward. This may have consequently over or underestimated the true value of transit time and subsequently the value of PWV. It may be that more phases are required in Cine-PC to generate a smoother velocity-time curve to facilitate the identification of the foot of the wave.

There are no general consensus as to which is the most methodologically robust way to determine the foot of the wave. Methods that have been used include the intersecting tangents, maximum upstroke of the second derivative, 10% upstroke, half maximum and the sigmoid method (134-136,138,140-142,150,152,159-161). The commonest method that have been used regularly was the foot-to-foot method, also known as the intersecting tangents method. Most authors have applied a regression line to the early systolic upstroke of the wave to intersect with a horizontal line that represents the baseline. The transit time is then calculated by the difference of the intersection of the tangent and the horizontal line between the two pulse waves. Some authors have applied a regression line to the entire systolic upstroke instead of the early section only. This could introduce error in cases where reflection wave may have distorted by the rapid upstroke of the systole.

There are several interesting similarities and differences when comparing our

study to others. Hardy et al (126) assessed the CCA PWV in three healthy volunteers between age of 25 and 50. The results showed PWV value of 6.4 m/s, 5.7 m/s and 7.4 m/s. They reported that the results were similar to those in the normal aorta and were consistent with the literature. Furthermore, they have also developed analysis tools to extract PWV semi-automatically. Comparing to our results, we found that aortic arch PWV was significantly higher than carotid PWV. In addition, we extracted PWV data manually. Although it was time consuming, it allowed us to scrutinise the quality of analysis at each step. We are aware of softwares that could perform PWV analysis automatically and perhaps it could be incorporated in our future studies. The methodology and results published by Keenan et al (127) was very similar to our study. They have recruited seven young healthy volunteers (age between 28 to 35) and PWV was measured over the carotid bifurcation with mean length of 54mm. The mean PWV was 5.1 m/s (range 3.3-8.5) with Chronbach's alpha of 0.77 on intra-scan repeatability. Kroner et al (128) recruited 13 healthy volunteers (mean age 25 ± 3) where PWV was assessed in the left carotid artery and the aortic arch. They found that the carotid PWV was significantly higher compared to aortic arch PWV (mean 5.8 ± 1 m/s versus mean 4.8 ± 0.7 m/s, $P<0.001$). This is consistent with normal physiology where impedance mismatch exists between central and peripheral arteries to allow incident wave reflection in young individuals. In contrary, we found that aortic arch PWV was significantly higher than carotid PWV. This could be explained by the difference in age group between the two studies and the increase in arterial stiffness with age in the aorta is more than in the peripheral arteries. Additionally, the aortic arch stiffness could also be

contributed by the presence of cardiovascular risk factors in our study. Even though the values of carotid PWV in these studies seemed to be similar to our results, a direct comparison cannot be made due to the small number of study participants.

We did not observe any significant association between age and aortic arch PWV in patients with carotid artery disease. Among blood pressure parameters and cardiovascular risk factors, only lower diastolic blood pressure and the presence of hypertension were significantly associated with higher aortic arch PWV. The inverse association between aortic arch PWV and diastolic blood pressure have been reported previously (162). This could be due to the early return of reflected waves secondary to increased arterial stiffness in the distal vasculature discussed in Chapter 1. Although several studies in the literature have shown an association between aortic and carotid PWV (130,163,164), we did not observe such relationship in our study. There are several factors that may explain the findings above. Studies on the association between carotid and aortic stiffness were mainly performed in healthy volunteers or patients without evidence of carotid stenosis. In contrast, we have measured carotid stiffness in patients with cardiovascular disease and evidence of carotid stenosis on imaging. Additionally, due to the presence of cardiovascular disease and other co-morbidities, our patients were at higher risk of having aortic atherosclerotic disease. These may have offset the relationship between aortic and carotid artery stiffness observed in other studies. Furthermore, we measured PWV in the aortic arch, not the full length of the aorta as we hypothesised that the aortic arch may be

physiologically closer to the carotid arteries in terms of the composition of the arterial wall. Furthermore, the heterogeneity in the methods of quantifying carotid and aortic stiffness in studies reported in the literature may have contributed to the discrepancy between our results and the others. Another important factor that should be considered was that the number of patients included in the analysis of aortic arch PWV was small. Interestingly, we found that the median PWV of the aortic arch was significantly higher than the carotid arteries. This was consistent with the normal physiology where aortic stiffness increases with age more than that of the carotid arteries and may partially explain the absence of relationship between carotid and aortic arch stiffness. Additionally, several studies have shown different results on the association between blood pressure parameters and arterial stiffness. A recent study by Benetos et al (162) found that systolic blood pressure did not demonstrate any significant increase whereas diastolic blood pressure was significantly decreased with age, resulting in increased pulse pressure. Moreover, only aortic but not peripheral PWV (carotid-brachial) was significantly increased with age (162). Paini et al (130) studied aortic and carotid stiffness in both normotensive and hypertensive patients and found that age was a major independent influence whereas the influence of mean blood pressure on carotid stiffness was only minimal. In addition to blood pressure parameters, the heart rate was thought to have an effect on arterial stiffness. However, this remains highly controversial. Sa Cunha et al (165) showed that raised heart rate was strongly associated with higher PWV even after adjustment for age and blood pressure. This was in contradiction to other

studies that did not observe such correlation (91,166).

To our knowledge, we have performed the first study to measure carotid and aortic arch stiffness using the same method. There are several limitations to this study. The issues regarding the Cine-PC sequence itself have been discussed earlier. In view of the novelty of this technique, perhaps we should have measured aortic PWV using plethysmography or applanation tonometry to validate our results. However, aortic MRI PWV had been validated before using invasive methods (121) and hence it may not be necessary for us to do so. In regards to the data that we acquired using Cine-PC, we could perhaps analysed PWV derived from flow-area curve (Q-A loop) for comparison. However, study has shown that Q-A derived PWV has poor correlation with the transit time method (the method that we have used) and hence the comparison may not be useful (167). Furthermore, it would be valuable to measure aortic PWV in healthy volunteers and compare it to patients with carotid artery stenosis. Moreover, inter-rater consistency could have been done to assess reliability of analysis. This was not performed due to the short time constrain of this study. In the next section, we shall briefly discuss the basic principles and methods of MRI detected carotid IPH.

3.5 Assessment of Magnetic Resonance Imaging of Carotid Plaque Haemorrhage

3.5.1 Introduction

MRI detected IPH (MRIPH) remains a relatively new technique that utilises the ability of MRI to exploit the differences in tissue make-up and display these as alterations in image contrast. This provides a unique opportunity to selectively discriminate between different tissues, both normal and pathological (168). It uses a T1 weighted sequence with fat suppression and nulling of signal from blood to detect presence of thrombus, which is represented as hyperintense signal.

The coagulation cascade is activated when the endothelium of the tunica intima or the fibrous cap of a carotid plaque is ulcerated or ruptured. This process involves a chain of reactions that lead to platelet aggregation, fibrin net and ultimately formation of thrombus. Haemoglobin that were trapped within the thrombus undergo oxidative denaturation from ferrous to the ferric form and hence become methaemoglobin. Methemoglobin is paramagnetic and is used as an endogenous contrast agent. It causes shortening of T1 and results in high signal on a T1-weighted acquisition (168).

There are several limitations of this technique. To be able to detect methaemoglobin successfully, it must be formed sufficiently and within a thrombus. Furthermore, imaging must be performed within days of

methaemoglobin formation as the hyperintense signal would be lost when it is converted to haemosiderin (168).

Early studies have demonstrated its application in diagnosing lower limb deep vein thrombosis (169) with a sensitivity and specificity of 96% and 90% (170) and Cohen's kappa coefficient of between 0.89 and 0.98 (170) respectively. This technique was extended to assess the severity of carotid atherosclerosis beyond the degree of intra-luminal stenosis by identifying carotid IPH. The presence of MRIPH has been demonstrated to identify unstable carotid plaque at high risk of recurrent ischaemic stroke (171-173) and promoting carotid plaque growth (174,175). A recent meta-analysis on MRIPH in the risk of recurrent ischaemic stroke demonstrated a significant predictive value of MRIPH for ipsilateral cerebral ischaemic events (OR 12·2; 95% CI 5.5 – 27.1) (173).

3.5.2 Magnetic Resonance Imaging of Carotid Plaque Haemorrhage

I. Protocol

The MRI technique used in this study to detect carotid IPH has previously been shown to be valid and reproducible (176,177). It uses a T1 weighted magnetisation prepared three dimensional gradient echo sequence (Cube) to detect methaemoglobin, which is an intermediate breakdown product of haemoglobin during clot formation. The sequence has been optimised to display haemorrhage, represented as a hyperintense signal with a suppressed (dark) background. It incorporates selective water-excitation pulse and the inversion time (T1) was chosen to null blood signal from the tissues. Fat and lipids are not excited as the excitation pulse is water only excitation pulse and the flowing blood is nulled by the inversion magnetisation preparation. Muscle and bone with their long T1 relaxation appear darker therefore suppressing the background signal. The images were acquired in sagittal plane.

All study participants were scanned using a 16-channel head, neck and spine coil. The following sequence was used to acquire carotid IPH in sagittal plane:

TR 920 ms, TE 11.5 ms, FA 90 degrees, interpolated voxel size 1x1x1 mm, acquisition matrix 288x288 with 100% FOV, acquisition time 420 s.

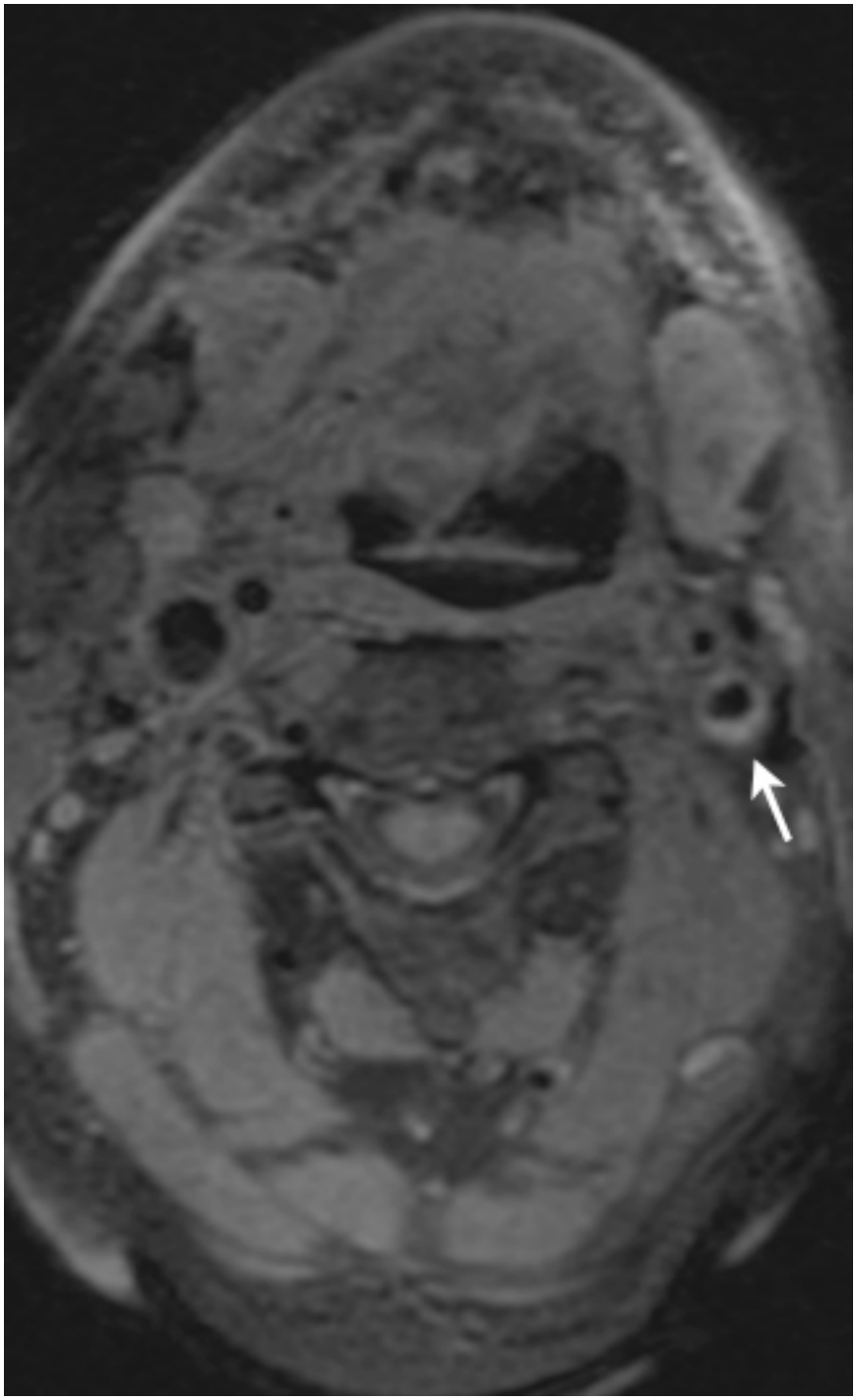


Figure 3.17: An example of an axial view of hyperintense signal representing intraplaque haemorrhage in the left ICA just distal to the carotid bifurcation.

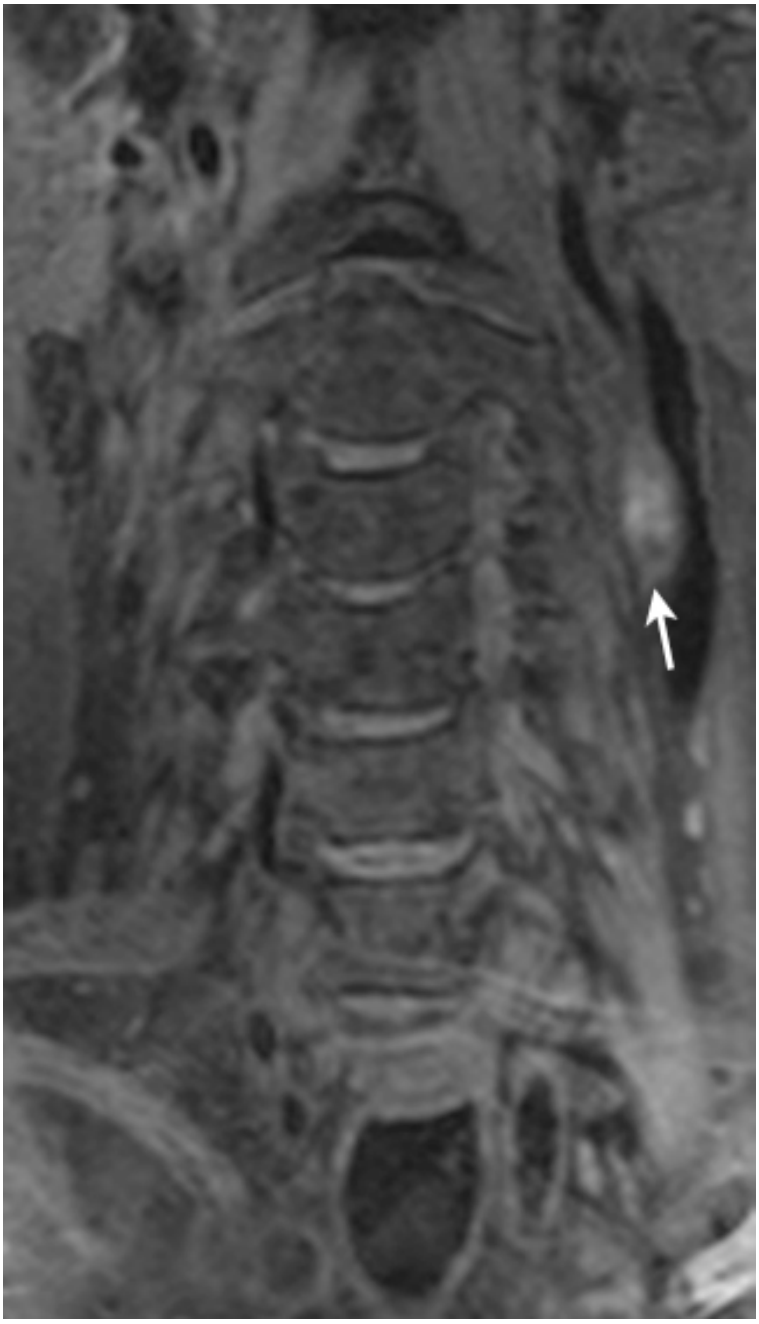


Figure 3.18: A coronal view of the same patient in Figure 3.17. The hyperintense signal in the left ICA represents intraplaque haemorrhage.

II. Post-Processing

Images were acquired in sagittal plane and reformatted in to axial series using multiplanar reconstruction within the workstation. Images were then written to an optical disc to be transferred and stored in the local secured server for analysis.

Image analysis for carotid IPH was performed off-line using JAVA Imaging (JIM) software provided by Xinapse System (www.xinapse.com).

The author underwent training of assessing MRI scans for carotid IPH by a clinical lecturer (NA) in the Division of Radiology and Imaging Science at the University of Nottingham.

III. Analysis of MRI IPH

The analysis of MRI IPH was performed using JIM. The software was installed and accessed via the secured server at the University of Nottingham.

The reformatted axial images were loaded on to JIM. This was followed by identification of the carotid arteries and the bifurcations. All areas of hyperintense (bright) signals suggesting haemorrhage within the carotid plaque were visually identified. This was performed within one centimetre above and below the carotid bifurcation. A region of interest (ROI) was drawn around the hyperintense area using threshold, irregular ROI option, which incorporates a seeding algorithm. The mean intensity of the ROI was noted (I_{car}). The ROI was then copied and placed on the adjacent sternocleidomastoid muscle. The mean intensity of the ROI on the muscle was noted (I_{mus}). The ratio of the intensity of the carotid plaque and the muscle was calculated (I_{car} / I_{mus}). Carotid plaque was considered plaque haemorrhage positive if the ratio was 1.5 or more. Carotid plaques with isointense or hypointense signal were considered to be plaque haemorrhage negative.

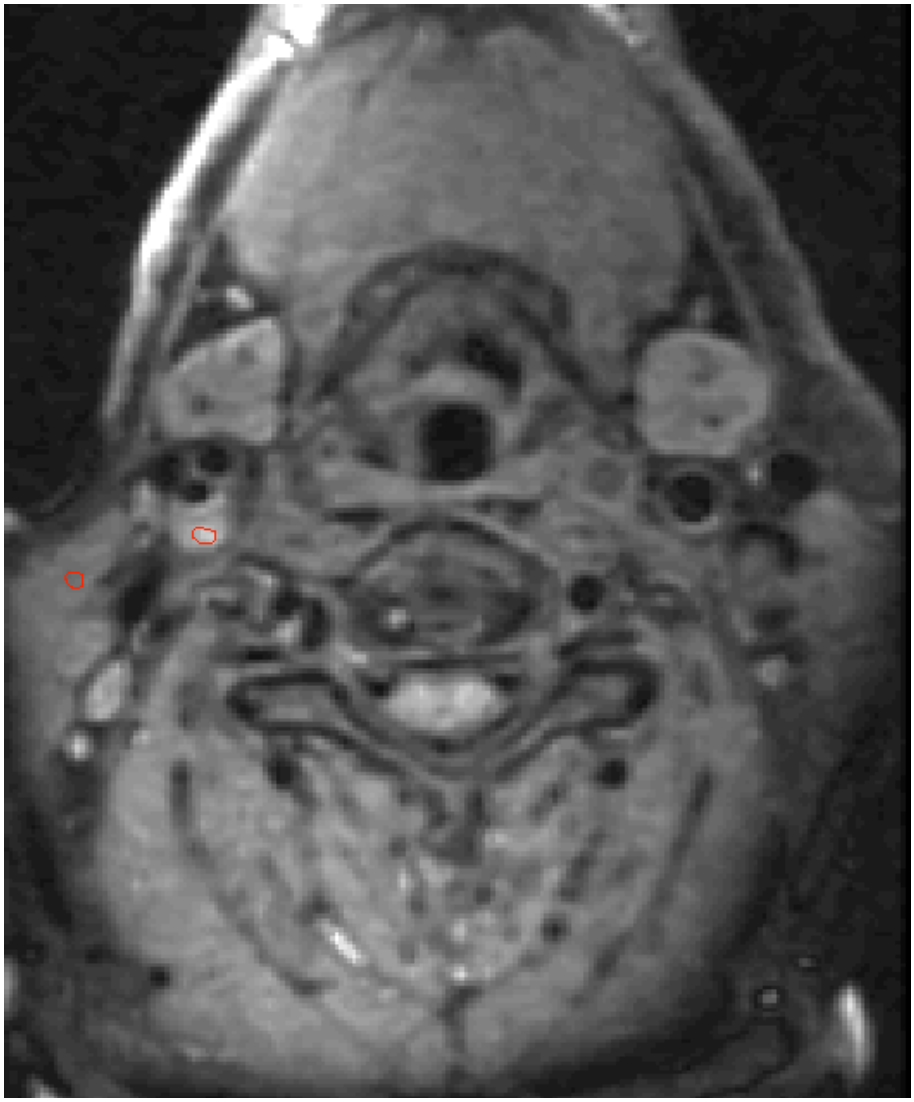


Figure 3.19: An example of determining the presence of intraplaque haemorrhage. ROI was first drawn around the hyperintense signal on the carotid plaque. A second ROI was drawn on the adjacent sternocleidomastoid muscle. The ratio of the intensity of the carotid plaque and the muscle was calculated (Icar / Imus). Carotid plaque was considered plaque haemorrhage positive if the ratio was 150% or more.

3.5.3 Quality Assessment

MRI carotid intraplaque haemorrhage (IPH) was assessed by two investigator (YPY and NA). Both investigators were blinded to the clinical history and the results of carotid PWV. MRI carotid IPH was assessed using JIM as described in Chapter 2.7.2 III. Hyperintense signals were visually identified one centimetre above and below the carotid bifurcation. An ROI was drawn on the hyperintense signal as well as the adjacent sternocleidomastoid muscle. The mean signal intensities were compared. Carotid IPH was considered present (MRI PH+) when the ratio of the intensity of the carotid plaque and the muscle (I_{car} / I_{mus}) was 1.5 or more. On the other hand, carotid plaques were considered to be absent of IPH (MRI PH-) if the ratio was less than 150% (isointense of hypointense).

I. Inter-rater consistency

29 patients underwent MRI of the carotid arteries. Two occluded carotid arteries were excluded from the analysis. A total of 56 carotid arteries were assessed for the presence of IPH. Table below summarises the number of carotid plaques with IPH according to whether patients were symptomatic or asymptomatic.

	MRI PH-	**MRI PH+**	**Total**
Asymptomatic	41	10	51
Symptomatic	4	1	5
Total	45	11	56

Table 3.6: Number of carotid plaque with the presence of intraplaque haemorrhage according to to whether patients were symptomatic or asymptomatic.

The inter-rater agreement was good between YPY and NA. The Cohen's Kappa value was 0.736 ($P<0.001$).

3.5.4 Discussion

The acquisition of MRI detected carotid intraplaque haemorrhage has previously been performed and validated at the University of Nottingham (172). However, previous acquisition of carotid MRIPH in this institution was performed axially rather than sagittally as in this study. Compared to MRI images acquired axially, the image quality of sagittal acquisition could be degraded by phase wrapping artefact. This became profound particularly in elderly patients where the shoulders are superimposed on the neck during scanning due to existing medical conditions such as osteoporosis. This may introduced bias during image analysis and partially explained the marginally lower Cohen's Kappa on inter-rater agreement compared to previous MRIPH studies performed at the University of Nottingham. We have attempted to overcome this issue by acquiring carotid MRIPH images axially on the GE Discovery MR750 3.0 Tesla but the acquisition time was doubled. Thus, in the author's opinion, future MRIPH imaging should be acquired axially in order to produce quality images to facilitate accurate image analysis.

In the next chapter, we shall discuss the results of carotid PWV of patients with carotid artery disease and healthy volunteers.

Chapter 4

Carotid Pulse Wave Velocity In Patients with Carotid Artery Disease and Healthy Volunteers

4.1 Introduction

The mechanism underlying the association between arterial stiffness and cardiovascular risk factors remains uncertain (178). Several observational studies that were discussed in Chapter 1 have investigated the association between aortic as well as carotid stiffness and acute ischaemic stroke. The outcomes of these studies were limited mainly by the heterogeneity in patient cohort and methods in assessing arterial stiffness.

Aortic stiffness has been shown to be an independent predictor for all cause and cardiovascular mortalities, fatal and non-fatal coronary events as well as fatal strokes in patients with uncomplicated essential hypertension, type 2 diabetes and end stage renal disease (7). The aims of this study were to assess carotid MRI PWV in patients with carotid artery disease and healthy volunteers. The association between carotid MRI PWV and the severity of carotid artery stenosis as well as blood pressure parameters were also explored.

4.2 Methods

4.2.1 Recruitment

Both symptomatic and asymptomatic patients as well as healthy volunteers were recruited for this study. The details of recruitment have been described in Chapter 3.2.

4.2.2 Clinical Assessment

All study participants were assessed by the author. The baseline characteristics were recorded using a standardised pro forma to identify any cardiovascular risk factors for stroke. The details of assessment have been described in Chapter 3.3.

4.2.3 MRI Pulse Wave Velocity

Following clinical assessment, all study participants underwent MRI of the carotid arteries and aortic arch. The MRI scan was performed with the Discovery MR750 3.0 Tesla (General Electric, Connecticut, USA) at the

University of Nottingham. PWV was acquired with Cine-PC with cardiac gating. The details have been described in Chapter 3.4.2.

4.2.4 Analysis of MRI PWV

Analysis of MRI PWV consists of two parts: the distance travelled by the pulse wave and the transit time. The distance was measured on the carotid artery ToF images. Further details have been described in Chapter 3.4.2 III.

4.2.5 Data Analysis

Statistical analyses were performed using IBM SPSS Statistics (Version 21; Chicago, Illinois). Categorical data were presented as absolute value and percentage. Continuous parametric data were presented as mean value with standard deviation whereas non-parametric data as median with interquartile range.

Categorical data were compared using Chi-square test, continuous non-parametric data with Mann-Whitney U and parametric data with unpaired t-test. $P<0.05$ was considered statistically significant.

Multiple linear regression was performed to examine the association between each baseline characteristic and carotid PWV, the relationship between MRI PWV and categories of the severity of carotid artery stenosis as well as aortic arch PWV. The results were then adjusted for age and blood pressure, which are known confounding factors of PWV.

PWV is known to correlate with age and blood pressure. A linear regression was performed to explore these two factors with the PWV results.

4.3 Results

4.3.1 Recruitment

A total of 45 patients were recruited in to the study. Of which, 29 (64%) were asymptomatic and 16 (36%) were symptomatic. In the asymptomatic group, two were excluded for having contraindication to MRI and a further two were excluded for being claustrophobic. In contrast, 12 patients were excluded in the symptomatic group. Two patients had contraindication to MRI, four patients underwent CEA the same day as being referred and six patients did not undergo MRI due to unavailability of the scanner or operator. 29 (29/45, 64%) patients (mean age was 71 ± 9.7 and 66% men) with 58 carotid arteries subsequently underwent MRI scan. Of these, three carotids were excluded: two had total carotid artery occlusion and one was unsuitable for analysis. A total of 55 carotid arteries were included in the analysis. Patient recruitment is summarised in Figure 4.1.

Eight healthy volunteers (63% male, mean age 72 ± 6) were recruited. All were hypertensive with no history of ischaemic heart disease, cerebrovascular disease or evidence of carotid artery stenosis on duplex ultrasound within the last 12 months. Table 4.1 summarises the baseline characteristics of both patients and healthy volunteers.

Due to the larger number of asymptomatic patients, multivariate linear regression analyses were performed with asymptomatic and symptomatic

patients together and separated (five carotid arteries were symptomatic). This was to examine if the inclusion of symptomatic patients have any effect on the results.

	PT n=29	HV n=8	P Value
Age	71 ± 10	72 ± 6	0.9
Male	66%	63%	1.0
Mean Systolic Blood Pressure (mmHg)	155 ± 20	148 ± 13	0.2
Mean Diastolic Blood Pressure (mmHg)	74 ± 11	74 ± 10	0.9
Mean Pulse Pressure (mmHg)	81 ± 17	74 ± 14	0.1
Mean Arterial Pressure (mmHg)	101 ± 12	99 ± 9	0.6
Mean Heart Rate	69 ± 15	68 ± 12	0.8
Chronic Obstructive Pulmonary Disease	18%	0	0.1
Diabetes Mellitus	36%	13%	0.1
Hypertension	81%	100%	0.1
Hypercholesterolaemia	86%	50%	0.006
Ischaemic Heart Disease	42%	0	0.002
Cerebrovascular Disease	71%	0	<0.001
Chronic Kidney Disease	9%	0	0.5

Table 4.1: Baseline characteristics of patients with carotid artery stenosis and healthy volunteers. PT=patients with carotid artery disease; HV=healthy volunteers.

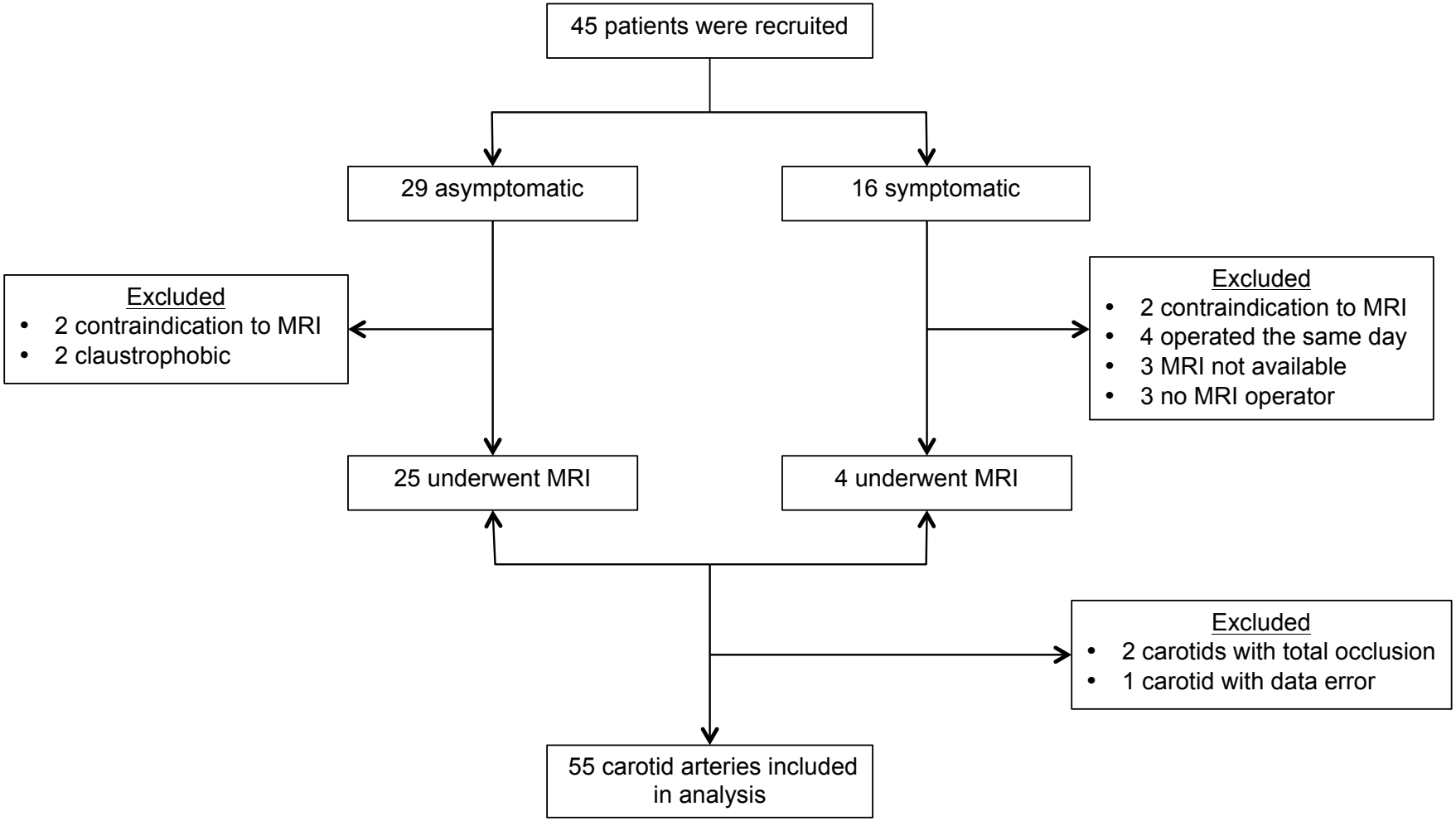


Figure 4.1: Flow diagram for patient recruitment.

4.3.2 Carotid Pulse Wave Velocity In Patients With Carotid Stenosis and Healthy Volunteers

Carotid PWV was compared between healthy volunteers and patients with severe (70-99%), moderate (50-69%) and no stenosis (<30%). There were no patients with carotid artery stenosis of 30-49%. Mann-Whitney U test was performed to compare the median PWV values between the groups. As shown in Table 4.2, carotid arteries with 50-69% and <30% stenosis had greater carotid PWV than those in healthy volunteers. However, the results were statistically insignificant.

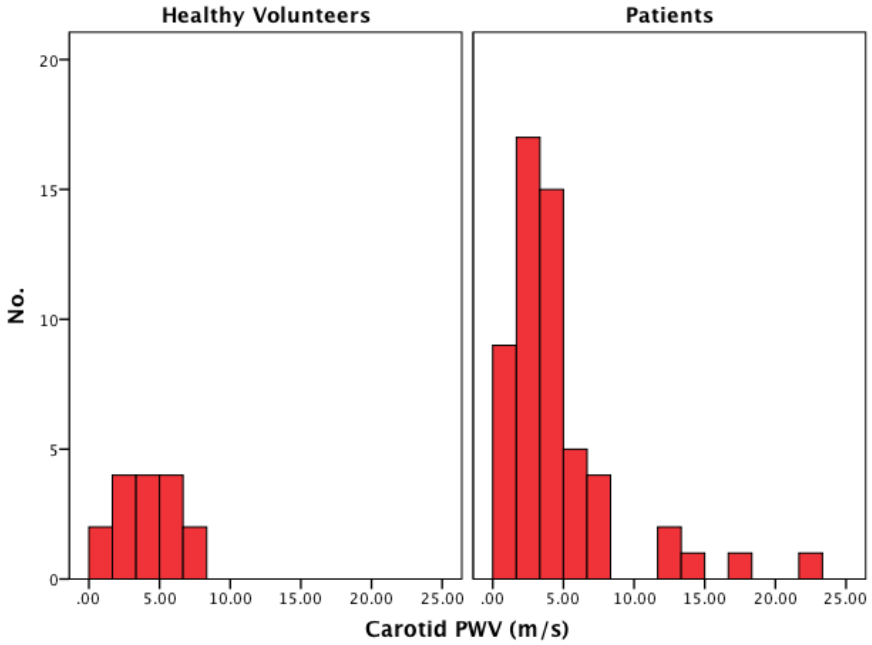


Figure 4.2: Carotid PWV was compared between patients with carotid artery stenosis and healthy volunteers.

Table 4.2 summarises the value of carotid PWV in healthy volunteers and patients with carotid artery stenosis categorised by severity. There were no statistically significant differences in the carotid PWV between the different groups of carotid stenosis and healthy volunteers.

	Carotid PWV (m/s, median with IQR)	**P value**
Healthy volunteers	4.1 (2.8-5.60)	
70-99% stenosis	3.5 (1.4-3.5)	0.07
50-69% stenosis	4.9 (2.5-5.3)	0.5
<30% stenosis	4.5 (2.3-5.8)	1.0

Table 4.2: Comparing carotid PWV between health volunteers and patients with different severity of carotid artery stenosis. There were no patients with 30-49% stenosis.

Multiple linear regression was used to examine the association between carotid PWV and age, blood pressure parameters as well as cardiovascular risk factors. Table 4.3 summarises the results where symptomatic and asymptomatic patients were analysed together whilst Table 4.4 summarises the results where symptomatic patients were excluded.

	R^2	P Value
Age (Categorical)	0.35	0.001
Age (Continuous)	0.009	0.4
Male	0.001	0.3
Mean Systolic Blood Pressure (mmHg)	0.047	0.07
Mean Diastolic Blood Pressure (mmHg)	0.007	0.5
Mean Pulse Pressure (mmHg)	0.038	0.1
Mean Arterial Pressure (mmHg)	0.028	0.1
Mean Heart Rate	0.019	1.0
Chronic Obstructive Pulmonary Disease	0.003	0.4
Diabetes Mellitus	0.003	0.4
Hypertension	0.006	0.3
Hypercholesterolaemia	0.018	0.9
Ischaemic Heart Disease	0.165	0.001
Cerebrovascular Disease	0.018	0.8
Chronic Kidney Disease	0.023	0.3

Table 4.3: The association between carotid PWV and age, blood pressure parameters as well as cardiovascular risk factors. The results of age was after adjustment for blood pressure parameters. Both asymptomatic and symptomatic patients were included in the analysis in this model.

	R^2	P Value
Age (Categorical)	0.30	0.006
Age (Continuous)	0.054	0.2
Male	0.001	0.9
Mean Systolic Blood Pressure (mmHg)	0.023	0.3
Mean Diastolic Blood Pressure (mmHg)	0.006	0.6
Mean Pulse Pressure (mmHg)	0.016	0.4
Mean Arterial Pressure (mmHg)	0.016	0.4
Mean Heart Rate	0.019	1.0
Chronic Obstructive Pulmonary Disease	0.004	0.7
Diabetes Mellitus	0.001	0.9
Hypertension	0.001	0.8
Hypercholesterolaemia	0.007	0.6
Ischaemic Heart Disease	0.21	0.001
Cerebrovascular Disease	0.017	0.4
Chronic Kidney Disease	0.024	0.3

Table 4.4: The association between carotid PWV and age, blood pressure parameters as well as cardiovascular risk factors. The results of age was after adjustment for blood pressure parameters. Symptomatic patients were excluded from the analysis in this model.

4.3.3 Association Between Carotid Pulse Wave Velocity and The Degree of Carotid Stenosis

Pulse wave velocity was measured in 55 carotid arteries. When carotid PWV was categorised according to the percentage of stenosis (90-99%, 80-89%, 70-79%, etc), patients with 50-59% stenosis was found to have the highest median value of PWV, 5.5 m/s (IQR 2.5-7.1). This was followed by patients with 30-39% stenosis, 4.2 m/s (IQR 3.7-4.2).

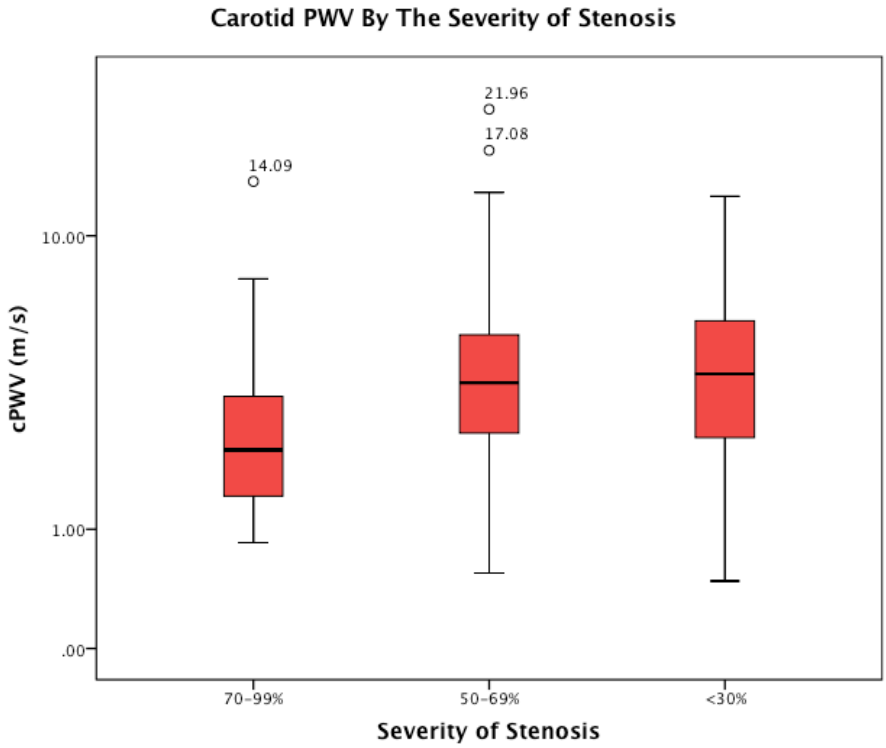


Figure 4.3: Carotid PWV categorised by the severity of stenosis. There were no patients in the group with mild stenosis (30-49%).

Multiple linear regression was performed to examine the relationship between carotid PWV and the degree of carotid artery stenosis (R^2=0.015, P=0.667). This was maintained after adjustment for age and blood pressure (R^2=0.055, P=0.943). When symptomatic patients were excluded from the analysis, the result was similar (R^2=0.031, P=0.5) even after adjustment for age and blood pressure (R^2=0.07, P=0.3).

4.3.4 Association Between Age and Blood Pressure Parameters with Carotid Pulse Wave Velocity

The mean ± standard deviation for age was 71 ± 9.7 years. Table 4.5 summarises the blood pressure parameters in 29 patients.

Bloods Pressure Parameters	Mean ± SD
Systolic Blood Pressure	155 ± 20 mmHg
Diastolic Blood Pressure	74 ± 11 mmHg
Pulse Pressure	81 ± 17 mmHg
Mean Arterial Pressure	101 ± 12 mmHg
Heart Rate	69 ± 15

Table 4.5: Mean blood pressure parameters in 29 study participants.

When symptomatic and asymptomatic patients were analysed together (Table 4.3), multiple linear regression analysis showed that increasing age group was associated with higher carotid PWV (R^2=0.333, P<0.0001). The results remained significant after adjustment for blood pressure parameters (R^2=0.35, P=0.001). However, when the analysis was repeated with age as a continuous variable, the association was not statistically significant (R^2=0.009, P=0.435). The results remained insignificant after adjustment for blood pressure parameters (R^2=0.054, P=0.07). On the other hand, linear regression analysis for carotid PWV and blood pressure parameters did not show any significant correlation (systolic blood pressure, R^2=0.047, P=0.07; diastolic blood pressure, R^2=0.007, P=0.5; pulse pressure, R^2=0.038, P=0.1; mean arterial blood pressure, R^2=0.028, P=0.1).

When symptomatic patients were excluded from the analysis, the results were very similar to the above where only categorical age groups and ischaemic heart disease were significantly associated with carotid PWV (Table 4.4).

Lastly, multiple linear regression analysis on carotid PWV and heart rate was not statistically significant (R^2=0.002, P=0.8). This was maintained after adjustment for age and blood pressure parameters (R^2=0.054, P=1.0).

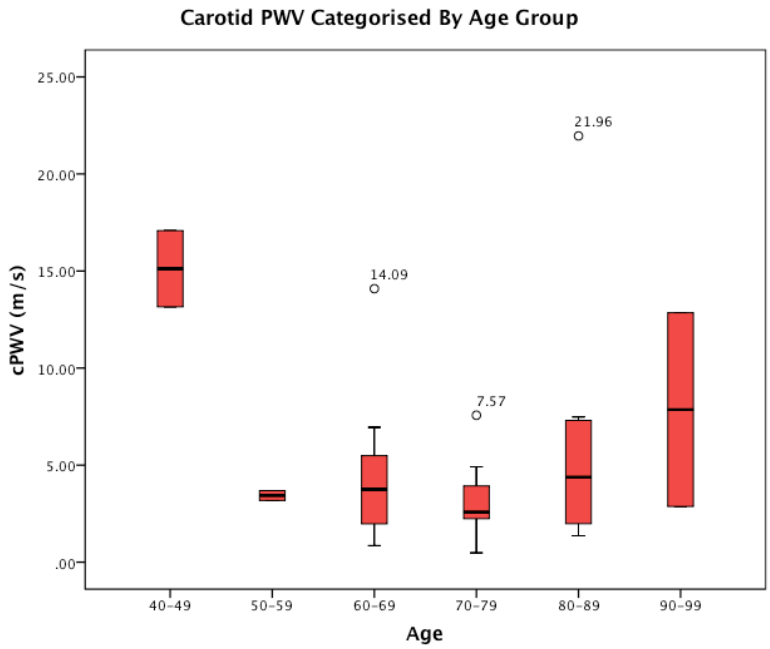


Figure 4.4: Carotid PWV categorised by age group after adjustment for blood pressure (R^2=0.35, P=0.001). Both asymptomatic and symptomatic patients were included.

4.4 Discussion

We did not observe any difference when comparing carotid PWV in patients with carotid artery stenosis and healthy volunteers. There are several possibilities that may explain our results. The number of healthy volunteers included in the study was low compared to the number of patients with carotid disease. Additionally, we did not have patients with carotid stenosis of 30-49% and it was difficult to speculate if this group of patients have higher or lower carotid PWV. These two factors may have introduced bias to the results.

We found that there was no correlation between carotid PWV and the severity of carotid artery stenosis. The question of whether atherosclerosis causes arterial stiffness or vice versa has long been debated and studies to date have shown conflicting results. Earlier published studies have shown a positive association between arterial stiffness and the severity of atherosclerosis (37,179-183). Nevertheless, several other studies did not observe such association (81,84,184-187). The conflicting results may be explained by the heterogeneity in the study design and methodology, in particularly the population of the study and methods used to quantify arterial stiffness.

We found that patients with 70-99% carotid artery stenosis had a trend of lower carotid PWV compared to patients with 50-69% and <30% stenosis. Although it may be true that there was no association between the severity of carotid artery stenosis and carotid PWV, the results could also be secondary to the inadequacy of temporal resolution in Cine-PC. This means that patients with high grade (70-99%) carotid artery stenosis could have had a higher

value of carotid PWV but the imaging sequence was not fast enough to pick up the high velocity signal in the arteries. This limitation has previously been discussed in Chapter 3.4.4.

The association between arterial stiffening and ageing has been well described in populations worldwide (7,188). Our results did show that carotid PWV was significantly associated with increasing age when the latter was categorised in to groups. The pathophysiology of increasing arterial stiffness with age was thought to be multi-factorial which include changes in extracellular matrix, endothelial dysfunction and activation of vascular smooth muscle cells. The details of these have been discussed in Chapter 1. Nevertheless, our results did not demonstrate any significant association between carotid PWV and blood pressure parameters obtained using automatic sphygmomanometer on the right brachial artery. No previous studies have examined the association between local PWV and blood pressure parameters measured at peripheral sites. The results observed could potentially be explained by the different sites where the two were measured. In the peripheral arteries, reflection sites are much closer than in central arteries leading to reflected waves traveling faster on peripheral arteries than on central arteries. Therefore, the amplitude of the pressure wave is higher in peripheral arteries than in central arteries based on the amplification phenomenon (7). The amplification phenomenon is maximal in young adults and may become minimal by middle age, especially with pathological ageing and increased arterial stiffness (188). This has been shown to be true when brachial and central pulse pressure were almost

identical in the observational Framingham population (189).

There are several limitations as to why our results on the association between carotid artery stiffness and the severity of atherosclerosis as well as blood pressure parameters cannot be compared directly to the results in the literature. Firstly, we have quantified carotid artery stiffness with novel technique of MRI PWV, which has not been validated before. Secondly, this study consists of a small number of study participants in which may limit the generalisability and the strength of the results. Thirdly, the heterogeneity of the study participants where the majority of them were patients with asymptomatic carotid artery stenosis. This has inevitably introduced bias and reduced the strength of the study. Our results may be drastically different if there were more symptomatic patients. Lastly, we did not take in to account the effect of calcification on arterial stiffness. Future studies should consider quantification of arterial calcification to adjust for derived PWV values.

In the final chapter, we shall discuss the association between carotid MRI PWV and MRI detected IPH.

Chapter 5

Association Between Carotid Pulse Wave Velocity and MRI Detected Intraplaque Haemorrhage

5.1 Introduction

Over the last decade, several studies have attempted to characterise carotid atherosclerotic plaque to identify vulnerable plaque using non-invasive imaging based on the evidence that certain histomorphological plaque features are associated with symptomatic carotid disease (190). Recent development of MRIPH has been studied extensively. A contemporary meta analysis showed that MRIPH independently and strongly predicts recurrent ipsilateral ischaemic events and stroke alone in symptomatic >50% carotid artery stenosis (173). However, about 40% of patients presenting with acute ischaemic stroke or TIA did not have evidence of MRIPH (171). This prompts the question whether there are other factors that predispose a vulnerable plaque to rupture. In the recent times, the prognostic value of PWV as a surrogate marker of arterial stiffness has been widely studied. Several observational studies have shown an association between arterial stiffness and stroke (36,80,111-120,123-125).

The aims of this study were to determine the association between carotid MRI PWV and MRIPH. In addition, we aimed to explore the association between carotid PWV in symptomatic and asymptomatic patients with carotid artery stenosis.

5.2 Methods

5.2.1 Recruitment

Both symptomatic and asymptomatic patients were recruited for this study. The details have been described in Chapter 3.2.

5.2.2 Clinical Assessment

All study participants were assessed by the author. The baseline characteristics were recorded using a standardised pro forma to identify any cardiovascular risk factors for stroke.

5.2.3 MRI Pulse Wave Velocity

Following clinical assessment, all study participants underwent MRI of the carotid arteries. The MRI scans were performed with the Discovery MR750 3.0 Tesla (General Electric, Connecticut, USA) at the University of Nottingham. PWV was acquired using cine phase contrast with cardiac gating. The details have been described in Chapter 3.4.

5.2.4 MRI Carotid Intraplaque Haemorrhage

MRIPH imaging was performed with the Discovery MR750 3.0 Tesla (General Electric, Connecticut, USA) at the University of Nottingham. The details of have been described in Chapter 3.5.

5.2.5 Data Analysis

Statistical analyses were performed using IBM SPSS Statistics (Version 21; Chicago, Illinois). Categorical data were presented as absolute value and percentage. Continuous parametric data were presented as mean value with standard deviation whereas non-parametric data were presented as median with interquartile range.

Categorical data were compared using Chi-square test and Mann-Whitney U test for continuous non-parametric data. $P<0.05$ was considered statistically significant and 95% confidence interval was reported.

To determine the relationship between MRI PWV and the presence of MRIPH, multiple logistic regression was performed. The results were adjusted for age and blood pressure, which are known confounding factors for PWV.

5.3 Results

A total of 29 patients with 58 carotid arteries underwent MRIPH imaging. Three carotid arteries were excluded: two had total occlusion and one had unsuitable data for MRI PWV analysis. Therefore, 55 carotid arteries were included in the analysis for the association between MRIPH and MRI PWV. Figure below summarises the number of carotid arteries with the presence of carotid IPH (MRIPH+).

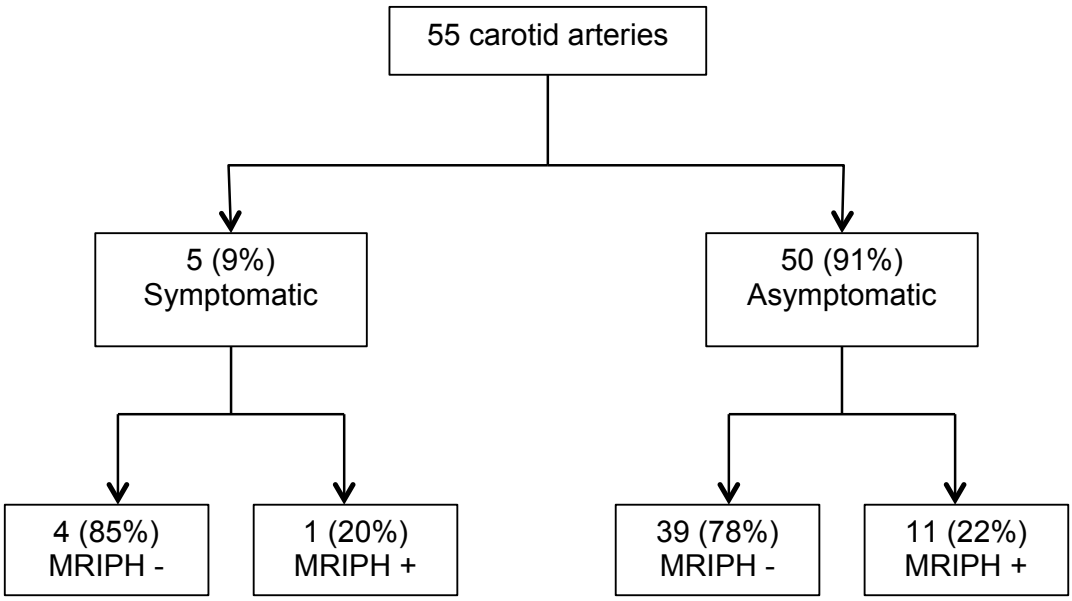


Figure 5.1: Numbers of carotid arteries according to MRIPH results. MRIPH+ = presence of MRIPH, MRIPH- = absence of MRIPH.

	Symptomatic n=5 (9%)	Asymptomatic n=50 (91%)	P value
Age (mean±SD)	68 ± 23	72 ± 8	0.7
Male	60%	66%	0.8
Body Mass Index	28 ± 1	26 ± 4	0.2
Mean Systolic Blood Pressure (mmHg)	138 ± 13	156 ± 20	0.06
Mean Diastolic Blood Pressure (mmHg)	71 ± 5	74 ± 11	0.9
Mean Pulse Pressure (mmHg)	67 ± 18	82 ± 16	0.09
Mean Arterial Pressure (mmHg)	94 ± 1	101 ± 13	0.08
Mean HR	71 ± 6	69 ± 15	0.4
Smoking	60%	24%	0.09
Chronic Obstructive Pulmonary Disease	0	20%	0.5
Diabetes Mellitus	0	40%	0.1
Hypertension	60%	40%	0.2
Hypercholesterolaemia	100%	84%	1.0
Ischaemic Heart Disease	0	46%	0.07
Cerebrovascular Disease	80%	70%	1.0
Chronic Kidney Disease	80%	10%	1.01
Anti-Platelet	100%	88%	1.0
Anti-Hypertensive	40%	68%	0.3
Statin	100%	72%	0.3
Anti-Hyperglycaemic	0	32%	0.3

Table 5.1: Patient demographics according to whether patients were symptomatic or asymptomatic.

	MRIPH + 12 (22%)	MRIPH - 43 (78%)	P value
Age (mean±SD)	73 ± 10	71 ± 10	0.5
Male	67%	65%	1.0
Body Mass Index	26 ± 3	27 ± 4	0.9
Mean Systolic Blood Pressure (mmHg)	159 ± 14	153 ± 21	0.4
Mean Diastolic Blood Pressure (mmHg)	74 ± 10	73 ± 12	0.7
Mean Pulse Pressure (mmHg)	85 ± 18	80 ± 17	0.3
Mean Arterial Pressure (mmHg)	103 ± 7	100 ± 13	0.2
Mean HR	66 ± 14	70 ± 15	0.3
Smoking	58%	35%	0.06
Chronic Obstructive Pulmonary Disease	0	23%	0.09
Diabetes Mellitus	33%	37%	1.0
Hypertension	92%	79%	0.4
Hypercholesterolaemia	67%	91%	0.06
Ischaemic Heart Disease	42%	42%	1.0
Cerebrovascular Disease	58%	74%	0.3
Chronic Kidney Disease	8%	9%	1.0
Anti-Platelet	100%	86%	0.3
Anti-Hypertensive	75%	63%	0.5
Statin	58%	79%	0.3
Anti-Hyperglycaemic	25%	30%	1.0

Table 5.2: Patient demographics according to MRI detected plaque haemorrhage.

The median carotid PWV in patients with presence (MRIPH+) and absence of MRIPH (MRIPH-) were compared. The results were 4.14 m/s (IQR 1.9-4.7) versus 3.0 m/s (IQR 1.9-4.7), P=0.09. This was shown in Figure 5.2.

Multiple logistic regression analysis demonstrated a positive association where carotid plaque with MRIPH+ had a higher value of carotid PWV (OR 1.17, 95% CI 1.0-1.36, P=0.04). Following adjustment for age and blood pressure parameters, the result became marginally stronger with OR 1.22, 95% CI 1.02-1.47, P=0.03. This was unchanged even after adjustment for the severity of carotid artery stenosis. This showed that the association between the MRIPH+ and higher carotid PWV was independent of the degree of carotid artery stenosis.

We repeated the multiple logistic regression above by excluding patients with high grade carotid stenosis (70-99%). This was due to the potential error of measuring carotid PWV in this group as discussed in Chapter 4.4. The results showed that MRIPH+ was still associated with higher carotid PWV with OR 1.15, 95% CI 0.98-1.36, P=0.086. Following adjustment for age and blood pressure parameters, the results showed OR 1.21, 95% CI 0.99-1.48, P=0.05. Table 5.3 summarises the results.

Further multiple logistic regression was performed to examine the association between symptomology (symptomatic versus asymptomatic) and carotid PWV. This showed a positive association between symptomatic carotid artery stenosis and higher value of carotid PWV with OR 1.4, 95% CI 1.0-1.7, P=0.005. When this was adjusted for age, systolic and diastolic blood pressure, the results showed OR 1.39, 95% CI 1.0-1.8, P=0.007.

Multiple Logistic Regression	OR	95% CI	P Value
Model 1	1.17	1.00-1.36	0.04
Model 2	1.22	1.02-1.47	0.03
Model 3	1.15	0.98-1.36	0.086
Model 4	1.21	0.99-1.48	0.05

Model 1 = All degree of carotid stenosis, unadjusted for age and BP

Model 2 = All degree of carotid stenosis, adjusted for age and BP

Model 3 = All except high grade stenosis, unadjusted for age and BP

Model 4 = All except high grade stenosis, adjusted for age and BP

Table 5.3: Multiple logistic regression to examine the association between MRIPH and carotid PWV.

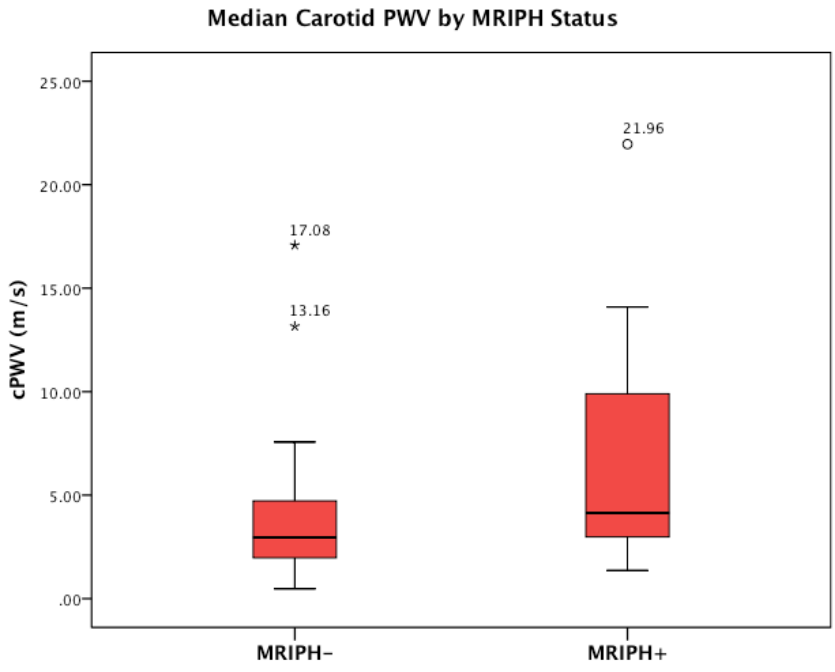


Figure 5.2: Carotid PWV in patients with MRIPH+ and MRIPH-.

5.4 Discussion

We have demonstrated a significant association between carotid PWV and MRI detected IPH. To our knowledge, this is the first study to investigate the association between these two. The positive association observed could be explained by neovascularisation of the carotid plaque and the mechanical forces exerted by the blood flow. The mechanical forces on the arterial wall have been discussed in Chapter 1.5.

Several studies have attempted to explained the pathomechanism of IPH ever since the presence of neovessels caused by angiogenesis have been suggested to be a potential source of haemorrhage (191-194). Neovascularisation of the carotid plaque have been established and confirmed in histologic studies as a consistent feature of vulnerable plaque in patients with cerebrovascular disease (194-196). Haemorrhage may occur at any stage in the evolution of plaque as observed by the presence of multiple haemorrhages of varying age contributing to the complexity of the plaque and its lipid content. Early studies have suggested that haemorrhage in to atheromatous plaques can been ascribed to three separate sources: (1) rupture of vessels in the base of the lesion derived from the vasa vasorum; (2) rupture of superficial and poorly supported vessels which could be originated from the lumen; and (3) dissection of blood from the arterial lumen through an ulcer or fissure (197). Indeed neovascularisation have been found in superficial and deep locations intimately related to both old and recent haemorrhage (194). It was believed that the rupture of these vessels

contributes significantly to IPH and subsequently thromboembolism. Besides neovessels, the network of vasa vasorum were found denser in symptomatic compared to asymptomatic patients (198). Furthermore, symptomatic patients had significantly more neovessels in plaques and fibrous cap (196,199,200). The majority of these vessels were found at the medial and lateral corners of the plaques with some being found at the base of the plaque (196). These neovessels were larger, more irregular and were associated with IPH. These highly immature vessels were largely devoid of smooth muscle cells which act as perivascular support. This suggests these might be the site where vascular leakage of blood in to the plaque occur leading to the expansion of necrotic core (201). Moreover, it was thought that the increase in neovessels in fibrous caps of symptomatic plaques could also be a possible vehicle for delivering inflammatory cells and macrophages to the fibrous cap to secrete growth and inflammatory factors as well as metalloproteases to promote plaque instability (196,202,203). In addition, the macrophages and lymphocytes may enter the plaque The level of vascular endothelial growth factors were also found to be elevated in immature vessels, suggesting a potential source of angiogenic factor in vascular remodelling.

The pathomechanism of IPH with increased carotid PWV can be explained in terms of fluid haemodynamics. The Bernoulli's principle in flow haemodynamics states that fluid in motion possess energy by virtue of its velocity and its pressure (204). This explains an increase in the speed of the fluid occurs simultaneously with a decrease in pressure or a decrease in the fluid's potential energy. For example, in an experiment with a Venturi meter,

the flow in a tube with a converging end causes the pressure on the lateral wall (static pressure) to be reduced at the narrow section where the velocity is increased (204). However, in a curvilinear model, the pressure on the outer curvature is increased by virtue of the centrifugal force while the pressure on the inner curvature is decreased in direct proportion to the same centrifugal force (204).

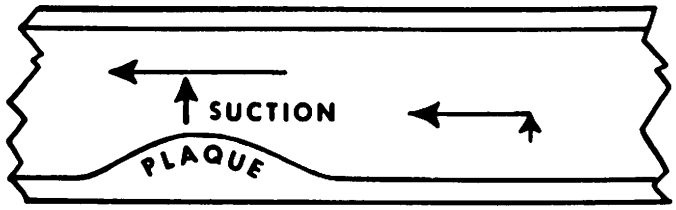


Figure 5.3: Velocity and pressure changes associated with atherosclerotic plaque (204).

Based on the principles of Bernoulli's effect, when blood flows through an atherosclerotic stenosis, the hydraulic force of the blood flow causes a decrease in pressure. This drop of pressure draws the plaque towards the center of the lumen (204). This has been proven in a study that investigated the effect of stenosis on wall motion and wall collapse using latex tube model (205). These forces that draw plaque towards the lumen may tear the neovessels, causing them to bleed in to the plaque. The other possible pathomechanism of IPH was the change in pressure between the intra-luminal area and the vasa vasorum. Carotid plaques contain networks of vasa vasorum originating from branches of the external or internal carotid artery

distal to the location of the plaque (206-208). The pressure in the vasa vasorum is normally lower than the luminal pressure. Blood may be diverted towards the area of lower pressure (in to the carotid plaque) when the luminal pressure of the stenosis is lower than that in the vasa vasorum due to Bernoulli's effect (204). Nevertheless, the question whether the pressure depression effect is enhanced by the severity of carotid stenosis or arterial stiffness remains debatable. In this pilot study, we did not find any association between the severity of carotid artery stenosis and arterial stiffness.

Several studies have supported the concept of Bernoulli's effect in the pathomechanism of IPH. Beach et al (209) studied the peak systolic velocities of the carotid arteries with ultrasonography in patients who were scheduled for CEA. They found that 80% of these patients had higher carotid peak systolic velocities when compared to patients without IPH. These patients had a carotid peak systolic velocities of >420cm per second, equivalent to a Bernoulli pressure depression of 70mmHg (209). A similar study by Mofidi et al (200) showed that peak systolic velocity was independently associated with the presence of IPH (OR 1.04, 95% CI 1.01-1.06, P<0.001). Furthermore, there was a strong correlation between peak systolic velocity and the quantity of IPH independent of the angiographic degree of ICA stenosis (R^2=0.68, P<0.0001). While peak systolic velocity at carotid stenosis has been shown to be associated with IPH, Binns et al (205) found a relationship between arterial wall motion and the degree of carotid stenosis. In their study using latex tube model, they found the increasing degree of stenosis progressively decreases the external pressure necessary to produce wall collapse. This was measured

at 37mmHg with 0% stenosis to 24mmHg with 81% stenosis (205). Hence, the negative pressures developed from the high velocities of systole tend to cause collapse of the vessel just distal to the stenosis with re-expansion during diastole. Recently, Selwaness et al (210) studied the association between arterial stiffness measured by PWV and carotid IPH. Although it was not clear whether the carotid or aortic PWV was measured, they have demonstrated that PWV was associated with the presence of IPH after adjustment for age, gender, mean arterial pressure, heart rate and traditional cardiovascular risk factors (OR 1.19, 95% CI 1.03-1.39). Although the significance was not reported, their results were very similar to our observations in this study.

Several studies have used computational methods to analyse the association between neovascularisation, plaque haemorrhage and local mechanical forces. Teng et al (211) studied the digitalised histopathological image of carotid plaque removed following CEA. They found the local maximum stress and stretch were greatest around neovessels with evidence of haemorrhage. Neovessels surrounded by red blood cells underwent a much larger stretch during systole and much larger stress and stretch variations during the cardiac cycle (211). Supporting the above findings, Teng et al (212) performed a three dimensional mechanical analysis of carotid plaque using reconstructed MRI images. It was found that the critical mechanical stretch was significantly higher in carotid plaques with evidence of juxtaluminal haemorrhage than those without. Moreover, the variation of stress and stretch of carotid plaque was significantly higher in this group (212). Huang et al (24)

examined the MRI carotid plaque with plaque haemorrhage using fluid-structure interaction study. The mean plaque wall stress and flow shear stress value from all haemorrhagic areas of the carotid plaques combined was higher than that from non-haemorrhagic areas. They concluded that plaque wall stress has a better correlation than flow shear stress with plaque haemorrhage. Sadat et al (213) studied carotid plaque with MRI and biomechanical stress analysis. They found that the maximum critical stress of symptomatic plaques was significantly higher than the asymptomatic plaques and those with recurrent TIA had a significantly higher plaque stress than those who suffered only a single episode..

The main limitation of this study were the small number of symptomatic patients compared to asymptomatic ones, which resulted in a smaller number of carotid plaques with the presence of MRIPH. This mismatch may have biased the results observed. Although every effort had been made to recruit symptomatic patients, the fast track surgical pathway and MRI scanner availability at short notice have made recruitment of symptomatic patients challenging. A larger number of symptomatic patients would be ideal as the incidence of MRI detected IPH would be higher. This would increase the power of the study and a more meaningful results. Furthermore, the image quality of MRIPH may have been affected by phase wrap artefact due to the sagittal acquisition technique. This has previously been discussed in Chapter 3.5.4.

Conclusions

We have demonstrated the feasibility of assessing local PWV using MRI over a short segment of an artery in this pilot study. It utilises the intrinsic flow sensitivity of MRI to quantify flow and velocity over a cardiac cycle. The acquisition time was very short (60 to 90 seconds) and can be easily incorporated in to any MRI protocol. Nevertheless, it remains a relatively new technique in assessing arterial stiffness and hence our findings should be regarded as preliminary results only. Furthermore, we found that the image quality of MRIPH acquired sagittally was compromised by aliasing artefact, which subsequently affect the accuracy of plaque haemorrhage analysis.

When we compared carotid PWV in patients with carotid artery stenosis and healthy volunteers, we did not observe any significant difference. In addition, there was no association between carotid PWV and the severity of carotid stenosis as well as blood pressure parameters. However, we did find that increasing age and patients with symptomatic carotid stenosis were significantly associated with higher carotid PWV after adjusting for age and blood pressure.

We examined the MRIPH status in patients and found that MRIPH+ carotid arteries have a significantly higher carotid PWV after adjusting for age, blood pressure and the severity of carotid artery stenosis. The association was maintained even after exclusion of high grade carotid stenosis due to the potential error in image acquisition in this group.

There are several limitations that should be taken in to account when interpreting our results. MRI PWV of the carotid arteries has not been validated previously. The validation of this new technique could be challenging

due to ethical and technical reasons as described in Chapter 3.5.4. Secondly, the sample size of this study was small and the results should be interpreted with caution. The majority of patients included were those with asymptomatic carotid artery stenosis. This was due to the fast track pathway in the management of symptomatic carotid artery stenosis and logistic problems in organising MRI scan at short notice. This has subsequently resulted in a small number of MRIPH+ carotid arteries. Thirdly, there were technical difficulties in determining the transit time between the pulse waves due to wave reflection in early systole and noise in the velocity-time curve. Finally, the number of age and hypertension matched healthy volunteers was small resulting in valid comparison to be inconclusive.

In our opinion, the study of local PWV with MRI remains in its infancy period. The areas that need further development include the MRI sequence itself, the validation of the technique and larger studies to examine its applicability. In regards to the sequence, a higher temporal resolution is needed to increase the sampling rate in order to acquire higher velocity signal such as those in high grade carotid artery stenosis. A higher spatial resolution is also desired to improve the accuracy in defining the region of interest during data analysis. Other techniques such as Fourier velocity encoding and 4D flow have been suggested to be superior to Cine-PC. However, these techniques are still being developed. The test-retest repeatability may be refined when these issues have been addressed. The next step in the study of PWV would be to validate the results once we are confident with the preciseness of this MRI technique. As discussed earlier, the most feasible method at present may be

to measure pressure and velocity in the carotid arteries using intra-arterial catheter during carotid stenting procedures. In order to fully assess the applicability of carotid MRI PWV, a longitudinal study with at least short term follow-up is needed. A larger number of patients with symptomatic carotid artery stenosis should be recruited to increase the prevalence of MRIPH+ carotid plaque. This would allow us to study the effect of plaque growth on carotid MRI PWV and its association with established markers of vulnerable plaque. Additionally, a larger number of age and hypertension matched healthy volunteers without imaging evidence of carotid artery disease should be recruited as control. This would allow us to re-examine the difference of carotid artery stiffness between the two groups of study participants. The main obstacles in measuring PWV using MRI were its dependency on age and blood pressure, in which an increase in either factors increases PWV value. The concern with blood pressure is that it may fluctuate during image acquisition and hence adding bias to the true value of the results. However, we found that between-subjects blood pressure have a higher variability than those within-subjects. Controlling blood pressure between subjects may be done with pharmacological agents. It would be interesting to do so within subject as well to examine the influence of blood pressure on PWV. However, ethical approval in this aspect may be challenging. Controlling the effect of blood pressure for the study of PWV will be difficult and we may well have to accept its confounding effect and adjust for it accordingly in multivariate analysis.

If carotid PWV does predict the unstable carotid plaque, it could improve our

knowledge in the natural history of carotid artery disease and subsequently influence our clinical management. Moreover, it could also become a marker for monitoring local arterial stiffness following pharmacological therapy. Identifying the vulnerable carotid plaque could improve our risk stratification strategy and a more targeted surgical and medical intervention to those who are truly at higher risk of recurrent ischaemic stroke.

Future Work

Background

Carotid artery intraluminal stenosis is a well established risk factor for acute ischaemic stroke. However, results from large randomised controlled trials have showed that it is not a sensitive predictor for recurrent events (1-4). Several other factors have since been investigated in search for a better prognostic tool. Our recent pilot study has showed an increased carotid artery stiffness as quantified by MRI PWV was associated with the presence of MRI detected IPH. The latter has been shown to be a more sensitive marker for carotid plaque instability compared to the severity of stenosis in a recent meta-analysis (173).

Research Questions

(1) Is higher carotid MRI PWV associated with acute ischaemic stroke?

(2) Is higher carotid MRI PWV associated with silent cerebral infarct?

Methods

Cohort study with prospective recruitment of study participants with at least unilateral asymptomatic carotid artery stenosis of >50%. Healthy volunteers are age and hypertension matched with no evidence of carotid artery stenosis on ultrasound. Both groups are follow-up for one year. Carotid PWV is assessed with MRI. Carotid artery flow velocity is measured with ultrasound to

validate carotid PWV. The primary end-points are evidence of acute ischaemic stroke or silent cerebral infarct on CT or MRI and death. Evidence of silent cerebral infarct on MRI is defined by the presence of silent lacunar infarct or white matter hyperintensity lesions (WMH). Furthermore, cerebral vascular reserve is measured with MRI arterial spin-labelling.

With the incidence of ischaemic stroke being 20% in asymptomatic carotid stenosis and <5% in healthy volunteers, 75 study participants and healthy volunteers are needed respectively in order to achieve power study of 80% (α=0.05, β=0.2).

Statistical Analysis

Carotid PWV are compared between patients and healthy volunteers as well as between higher and lower values with analysis of variance (ANOVA). Multiple logistic regression with adjustment for age and blood pressure parameters to determine the risk of ischaemic stroke and death in groups with higher and lower carotid PWV.

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Appendix

1 - Confirmation of REC Approval

NHS
National Patient Safety Agency

National Research Ethics Service

NOTICE OF SUBSTANTIAL AMENDMENT

For use in the case of all research other than clinical trials of investigational medicinal products (CTIMPs). For substantial amendments to CTIMPs, please use the EU-approved notice of amendment form (Annex 2 to ENTR/CT1) at http://eudract.emea.eu.int/document.html#guidance

To be completed in typescript by the Chief Investigator in language comprehensible to a lay person and submitted to the Research Ethics Committee that gave a favourable opinion of the research ("the main REC"). In the case of multi-site studies, there is no need to send copies to other RECs unless specifically required by the main REC.

Further guidance is available at http://www.nres.npsa.nhs.uk/applicants/review/after/amendments.htm.

Details of Chief Investigator:

Name:	Mr Nishath Altaf
Address:	Department of Vascular Surgery Queens Medical Centre Nottingham NG7 2UH
Telephone:	0115 924 9924
Email:	nishaltaf@gmail.com
Fax:	

Full title of study:	Non-invasive assessment of high risk carotid plaque using Magnetic Resonance Imaging: Comparison with ultrasound and correlation with risk of cerebral thromboembolism and infarction
Name of main REC:	Nottingham Research Ethics Committee 2
REC reference number:	REC/Q2120209
Date study commenced:	18/11/11
Protocol reference *(if applicable)*, current version and date:	Version 7
Amendment number and date:	17

Type of amendment (indicate all that apply in bold)

(a) Amendment to information previously given on the NRES Application Form

Yes

If yes, please refer to relevant sections of the REC application in the "summary of changes" below.

(b) Amendment to the protocol

Yes

If yes, please submit either the revised protocol with a new version number and date, highlighting changes in bold, or a document listing the changes and giving both the previous and revised text.

(c) Amendment to the information sheet(s) and consent form(s) for participants, or to any other supporting documentation for the study

Yes

If yes, please submit all revised documents with new version numbers and dates, highlighting new text in bold.

Is this a modified version of an amendment previously notified to the REC and given an unfavourable opinion?

No

Summary of changes

Briefly summarise the main changes proposed in this amendment using language comprehensible to a lay person. Explain the purpose of the changes and their significance for the study. In the case of a modified amendment, highlight the modifications that have been made.

If the amendment significantly alters the research design or methodology, or could otherwise affect the scientific value of the study, supporting scientific information should be given (or enclosed separately). Indicate whether or not additional scientific critique has been obtained.

We would like to request 2 amendments to the study protocol.

1. Measuring pulse wave velocity (PWV) of the carotid artery using 3T MRI
Pulse wave velocity is the speed of blood flowing through a vessel. It is a direct measurement of stiffness of the artery. It is calculated by obtaining the length of the carotid artery (distance) divided by the time taken for blood flowing through it (time). Higher PWV means the artery is unable to distend as blood flowing through, therefore stiffer. Studies have demonstrated the association between increased carotid PWV and stroke [1,2].

Although increased aortic PWV has also been shown to relate to stroke, but studies have demonstrated that it is only moderately correlated with carotid stiffness and carotid stiffness may be a more specific predictor of stroke [3]. Our previous study [4] has demonstrated MRI detected carotid plaque hemorrhage (PH) was a significant risk factor predicting recurrent symptoms in patients with symptomatic carotid stenosis and that it was able to identify those 'active' carotid plaque that continued to embolise. However, only about half the patients with PH positive in the study had embolisation. This suggests additional factor(s) may play a role in triggering embolisation. By measuring carotid PWV with this novel technique, we hope to study the link between carotid arterial stiffness and PH.

2. Inclusion of healthy volunteer to establish scanning protocol using 3T MRI

Although measuring carotid PWV with MRI has been demonstrated to be feasible by another research group [5], it was done using a lower resolution scanner and different sequence. Therefore, we aim to establish a protocol in measuring carotid PWV using 3T MRI by scanning healthy volunteers who have not experienced any symptoms of stroke.

Once a scanning protocol is in place, we intend to recruit about 20 participants with symptomatic carotid stenosis to participate in a pilot study over the next 12 months.

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Any other relevant information

Applicants may indicate any specific ethical issues relating to the amendment, on which the opinion of the REC is sought.

None

List of enclosed documents

Document	*Version*	*Date*
Study Protocol	7	18/11/11
Patient Information	8	18/11/11

Declaration

- I confirm that the information in this form is accurate to the best of my knowledge and I take full responsibility for it.
- I consider that it would be reasonable for the proposed amendment to be implemented.

Signature of Chief Investigator: .....

Print name: NISHA TUR ACOTT

Date of submission: 15/11/..

NHS
Health Research Authority

NRES Committee East Midlands - Nottingham 2
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Tel:
Fax:

04 January 2012

Mr Nish Altaf
Nottingham University Hospitals NHS Trust
Division of Academic Radiology
School of Clinical Sciences
B Floor , west Block
Queen's medical Centre
Derby Road, Nottingham
NG7 2UH

Dear Mr Altaf

Study title: **Non-Invasive Assesment of high risk plaque using magnetic resonance imaging : Comparison with ultrasound and correlation with risk of cerebral thromboembolism and infection.**
REC reference: **Q2120209**
Amendment number: **N/A**
Amendment date: **18 November 2008**

The above amendment was reviewed at the meeting of the Sub-Committee held on 19 December 2011.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Participant Information Sheet	8	05 November 2011
Protocol	7	04 November 2011
Notice of Substantial Amendment (non-CTIMPs)	N/A	18 November 2008
Participant Consent Form	Healthy Volunteer 1	02 January 2012
Participant Information Sheet	Healthy Volunteer 1	02 January 2012

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

Q2120209:	Please quote this number on all correspondence

Yours sincerely

pp **Dr Martin Hewitt**
Chair

E-mail: stephen.briggs@nnotts.nhs.uk

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Paul Cartledge, University of Nottingham, University Park, Nottingham. NG7 2RD*

Mr Yao Pey Yong, Research Fellow in Vascular Surgery and Academic Radiology, Divison of Academic Radiology, School of Clinical Sciences, B Floor West Block, Queens Medical Centre, Derby Road, Nottingham. NG7 2UH

NRES Committee East Midlands - Nottingham 2

Attendance at Sub-Committee of the REC meeting on 19 December 2011

Name	*Profession*	*Capacity*
Dr Martin Hewitt	Consultant Paediatric Oncologist	Expert
Ms Margret Vince	Translator	Lay Plus

Also in attendance:

Name	*Position (or reason for attending)*
Mr Stephen Briggs	Assistant Co-ordinator
Miss Heather Harrison	Committee co-ordinator

2 - Confirmation of R&D Approval

Nottingham University Hospitals NHS
NHS Trust

Mr Yao Pey Yong
Research Fellow in Vascular Surgery and Academic Radiology
B Floor West Block
Queen's Medical Centre
Derby Road
Nottingham NG7 2UH

Email: yaopey@doctors.org.uk

17th January 2012

Research Development
E11 Curie Court
Queen's Medical Centre Campus
Derby Road
Nottingham NG7 2UH
Tel: 0115 970 9049

Dear Sir/Madam,

ID: (Q)DI010301 **Non-invasive assessment of high risk carotid plaque using Magnetic Resonance Imaging: Comparison with ultrasound and correlation with risk of cerebral thromboembolism and infarction**

I am writing on behalf of Mr N Altaf who is the lead investigator of this study.

We have recently applied for ethical substantial amendment for our study and it has been approved (REC reference: Q2120209). I am therefore writing to apply approval from the R&D.

Attached are all the documents approved by REC:

Document	Version
Study Protocol	7
Participant Information Sheet	8
Healthy Volunteer Information Sheet	1
Healthy Volunteer Consent Form	1
Letter of Substantial Amendment	17
Substantial Amendment Approval Letter	N/A

Thank you.

Yours sincerely,

Mr Yao Pey Yong
Research Fellow in Vascular Surgery and Academic Radiology

Nottingham University Hospitals NHS Trust

Please reply to:
Research and Innovation
Nottingham Integrated Clinical Research Centre
Research and Development
C Floor, South Block
Nottingham University Hospitals NHS Trust
QMC Campus

Telephone: 0115 970 9049
Fax: 0115 849 3295
E-mail: brian.thomson@nottingham.ac.uk

20 February 2012

Mr N Altaf
Academic Radiology
B Floor West Block
Queens Medical Centre
Nottingham
NG7 2UH

Dear Mr Altaf

ID: (Q)DI010301* **Non-Invasive Assessment of High Risk Carotid Plaque Using Magnetic Resonance Imaging: Comparison with Ultrasound and Correlation with Risk of Cerebral Thromboembolism and Infarction**

Thank you for informing R&I of the following amendments:

. Protocol, version 7, 04/11/11.
. Participant Information Sheet, version 8, 05/11/11.
. Healthy Volunteer's Information Sheet, version 1, 03/01/12.
. Healthy Volunteer's Consent Form, version 1, 03/01/12.
. Notice of Substantial Amendment, 18/11/08.

R&I approval of the amendment is subject to the NUH header and relevant contact details being added to the study paperwork where appropriate.

The amendment has been given R&I approval, however, you may be contacted in due course if we wish to re-visit the original costings attached to the study.

Yours sincerely

Dr Maria Koufali
Deputy Director of Reseach and Innovations

3- Participant Information Sheet

Nottingham University Hospitals NHS

PARTICIPANT INFORMATION SHEET

Study Title: Non-invasive Assessment of High-Risk Carotid Plaques using Magnetic Resonance Imaging, ultrasound and correlation with the risk of cerebral thromboembolism and infarction

REC reference No: Q2120209

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with your friends and relatives if you wish. Ask us if there is anything that is not clear or if you will like more information. Take time to decide whether or not you wish to take part.

What is the purpose of this study?

Fatty deposits causing narrowing of the neck arteries taking blood to the brain (carotid arteries) are a common cause of stroke. **People who have symptoms of stroke or mini-stroke (TIA) and some people who have not had any symptoms (asymptomatic) but have a narrowing (> 60%) of the carotid artery are offered an operation called a carotid endarterectomy to remove the fatty deposits**. This operation reduces the chances of having a stroke in the future that might leave them permanently disabled. The operation itself does carry a small risk of stroke, and it is important to remember that not everybody with narrowed carotid arteries will go on to have a stroke if they do not have an operation. So if we had a better way of telling who was at highest risk we might be able to cut down the number of people who have the operation and target only those high risk patients. Also some people have very bad stroke as their first ever symptom of a carotid artery problem. It is then too late to offer them an operation to prevent them becoming disabled. If we had better ways of telling which arteries were likely to cause problems we could offer these people surgery to prevent stoke before any problems occurred.

This study is a continuation of our previous successful research to try to find better ways to tell which arteries in the neck are dangerous and need surgery

and which can be left alone. We have developed a test using Magnetic Resonance Imaging (MRI), which can identify bleeding inside the narrowed segment of the artery, which is thought to make the artery more likely to cause a stroke.

The main way in which strokes are caused is by little pieces of clot (emboli) breaking off from the artery and travelling up into the brain (embolisation). Our previous research showed that this happened more frequently in narrowed segment of artery that was bleeding. However, we have also identified that only about half of these patients who had bleeding had embolisation. This suggests that bleeding in the narrowed segment of the artery may not be the only factor that contributes to embolisation that causes stroke and factor(s) that have yet to be identified plays a role.

Recent studies by other researchers showed that stiffness of the carotid arteries may have an important role in causing bleeding in narrowed segment of the artery, thus causing embolisation and stroke. We can measure the stiffness of the carotid arteries by a technique called pulse wave velocity (PWV). It involves measuring the speed of the blood flowing through the carotid arteries using MRI. The higher the speed of blood flowing through means a stiffer artery. If our research confirms that stiffness of the neck artery does relate to bleeding in the narrowed segment, this might allow us to have a better way in identifying those at higher risk of stroke. This also means that patients who are at lower risk of stroke may or may not need surgery. This is an observational study which means we collect the results of your MRI from both your neck arteries and compare them to see if any relationships can be found.

What will be my participation in this study?

Your participation will involve routine ultrasound of your neck arteries and a MRI scan of your brain and neck arteries. It is a completely painless procedure and it will be done before your operation. This test will not affect your care after the operation. The MRI scan of your neck arteries and the brain will take about an hour altogether. During this scan we will also do a scan of your neck with our

test to assess the stiffness and look for bleeding in the narrowed segment of the carotid artery, which takes about 5-10 minutes.
The MRI scan will take place at the Queens Medical Centre, Nottingham. It will be done on a 3T MRI scanner (Philips Achieva 3T). 3T relates to the strength of the magnet in the scanner.

We will also like to perform two extra scans. One in the week before your operation, and one within two weeks after your operation. This will be to see whether there are any areas of your brain where that have been affected during your operation.

Why have I been chosen?
You have been chosen because you have a narrowing in your carotid artery and are suitable for a carotid artery operation to try to reduce your risk of stroke and because you have agreed to have this operation.

Do I have to take part?
It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

What do I have to do?
There are no lifestyle restrictions as a result of this study

What are the side effects of taking part?
We will assess whether you are safe to have an MRI scan by asking some specific questions. If you are safe to have an MRI scan then there should be no side effects.

Will my taking part in this study be kept confidential?
All information that will be collected about you during the course of this study will be kept strictly confidential. Any information about you that leaves the

hospital will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of this study?

The results will be available at the end of one years and will be published in medical journals and presented at national and international conferences. If you would like a copy of the publications, this can be forwarded to you after they have been published by contacting Dr Solomon Akwei at the telephone number below.

Who is organising and funding the research?

This study is organised and funded jointly by the Division of Radiology and Imaging Sciences, and the Department of Vascular and Endovascular Surgery, in conjunction with the University of Nottingham.
No profit will be made as a result of this study.

Will taking part in this study delay my treatment?

No.

Can I change my mind once I have decided to take part?

Yes, you can decide to leave the study at any time. It will not affect your treatment.

Thank you for your help and cooperation. Please call the number below if you have any further questions.

Mr Yao Pey Yong	Research Fellow
Mr Nishath Altaf	Specialist Registrar, Vascular Surgery
Dr Akram Hosseini	Research Fellow
Mr Shane MacSweeney	Consultant Vascular Surgeon
Professor Dorothee Auer	Professor of Radiology

If you have any enquiries please contact Dr Solomon Akwei at the Department of Vascular and Endovascular Surgery or The Division of Radiology and Imaging Sciences.
Queens Medical Centre: 0115 924 9924 or 0115 823 1176

Version 8. Date: 5th November 2011. Flesch Reading Ease 62.3, Flesch-Kincaid Grade Level 9.2

PARTICIPANT INFORMATION SHEET

4 - Study Pro Forma

Changes In Cerebral and Central Hemodynamics After Carotid Endarterectomy

Patient's Details

Subject No:

Date of Clinic:

Date of MRI:

Date of CEA:

Symptoms and Side:

Date of 1st Symptoms:

Date of Last Symptoms:

Risk Factors	Y / N	Duration	Additional Details
Smoking			Pack yrs:
COPD			
DM			Last HbA1C:
HTN			
HCL			
AF			
IHD			
MI		Last 6/12	
PVD			
Prosthetic valve			
Previous TIA/Stroke			
CKD			

Medications	Y / N Date Started	Dose
Aspirin		
Clopidogrel		
Dipyridamole		
Warfarin		
Alpha/Beta-Blocker		
ACEi		
Ca Blocker		
Diuretics		
Statins		
Insulin		
Oral Hypoglycaemics		

Changes In Cerebral and Central Hemodynamics After Carotid Endarterectomy

Height (m)	
Weight (kg)	
BMI	
SBP	
DBP	
HR	
Cardiac Echo (if done)	

% Stenosis	**Left**	**Right**
CCA		
ICA		
ECA		
VA		

Characteristics of Plaque	
Smooth	
Irregular	
Ulcerative	
Type 1 – Dominantly echolucent	
Type 2 – Substantially echolucent with small areas of echogenecity	
Type 3 – Dominantly echogenic with small areas of echolucency (<25%)	
Type 4 – Uniformly echogenic (equivalent to homogenous)	
Type 5 – Not defined	

Bloods Results	**Values & Date**
Hb	
Wcc	
Plt	
INR	
APTT	
Urea	
Crea	
Chol	

5 - Presentations and Publications

Presentations

(1) Poster presented at post-graduate research forum at the University of Nottingham in June 2013.

(2) Poster presentation accepted for European Society of Vascular Surgery Spring Meeting May 2014.

Publications - In writing

(1) Systematic review and meta-analysis of pulse wave velocity and stroke.

(2) Arterial stiffness in carotid artery disease: a new marker for carotid plaque instability.

Arterial Stiffness, Intraplaque Haemorrhage and Cerebral Haemodynamic in Carotid Artery Disease

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

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Background

- The degree of carotid stenosis has a low predictive value for the risk of recurrent ischaemic stroke secondary to carotid thromboembolic disease
- Carotid intraplaque haemorrhage detected by MRI (MRPH) has been shown to predict recurrent symptoms in patients with carotid stenosis[1]
- Pulse wave velocity (PWV) is a non-invasive method of assessing arterial stiffness. It has been shown to predict cardiovascular and cerebrovascular morbidity as well as mortality[2]
- Carotid artery stiffness may influence local arterial remodeling and promote plaque rupture

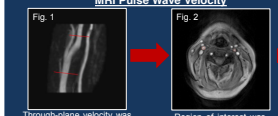
Objectives

- To investigate:
  - Correlation between degree of carotid stenosis and MRI pulse wave velocity (MRPWV)
  - Correlation between MRPH and MRPWV
  - To compare MRPWV and cerebral function (cerebral perfusion and cognitive function) between patients with carotid stenosis and healthy volunteer

Methods

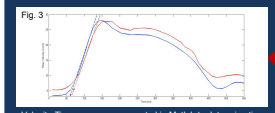
- Eligible criteria: carotid artery stenosis of >50% on duplex ultrasonography
- Patient demographics, baseline characteristics and blood pressure parameters were collected
- MRPH and MRPWV were assessed using MRI at 3 Tesla. Cerebral function was assessed by measuring cerebral blood flow with MRI arterial spin labeling (ASL) and the Montreal Cognitive Assessment (MoCA)

MRI Pulse Wave Velocity



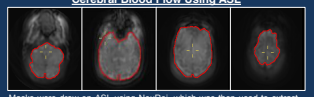
Through-plane velocity was acquired perpendicular to the carotid arteries

Region of interest was drew around the internal and common carotid arteries



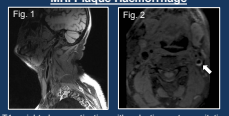
Velocity-Time curve was generated in Matlab to determine time delay between the two pulse waves

Cerebral Blood Flow Using ASL



Masks were drew on ASL using NeuRoi, which was then used to extract cerebral blood flow data manually using FSL

MRI Plaque Haemorrhage



T1 weighted magnetisation with selective water-excitation pulse and inversion time were used to null blood signal from the tissues. Haemorrhage was represented as hyperintense signal with a suppressed (dark) background

MoCA

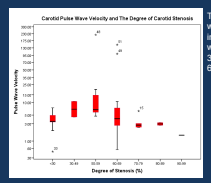
Validated 30-point based assessment tool to detect mild dementia

Results

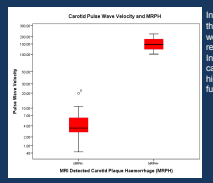
- 33 carotid arteries with >50% stenosis were assessed in 26 patients (17 male, median age 73 (IQR 65-77))

Blood Pressure Parameters	Mean ± SD
Systolic blood pressure	156 ± 18
Diastolic blood pressure	74 ± 11
Pulse pressure	83 ± 16
Mean arterial pressure	101 ± 12

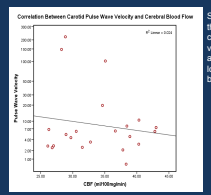
Blood pressure parameters of study participants



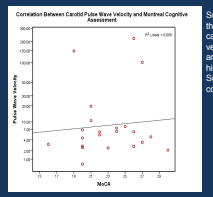
The median carotid pulse wave velocity was highest in the group of patients with carotid stenosis of 30-49%, 50-59% and 60-69%.



In patients with MRPH-, the values of carotid PWV were very similar to those reported in the literature. In those with MRPH+, carotid PWV values were high. The results need further clarification.



Scatter plot demonstrates that patients with higher carotid pulse wave velocity (i.e. carotid arterial stiffness) has a lower global cerebral blood flow.



Scatter plot demonstrates that patients with higher carotid pulse wave velocity (i.e. carotid arterial stiffness) have a higher score of MoCA. Score of 26 or above is considered normal.

Discussion

- Regional arterial stiffness (PWV) can be assessed using MRI
- There was no significant association between the degree of stenosis and MRPWV
- MRPWV in MRPH- were consistent with the literature, whereas those in MRPH+ needs further clarification

Conclusion

Carotid PWV may help to identify patients who are MRPH- but at high risk of plaque rupture. Further work is needed to compare carotid arterial stiffness and cerebral perfusion in healthy volunteer

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References
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2. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 2006;27(21):2588-605